

A PHYSIOLOGICAL STUDY OF ROOTING AND CALLUSING IN APPLE AND WILLOW

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CHARLES F. SWINGLE

Dissertation submitted to the Board of University
Studies of the Johns Hopkins University in
conformity with the requirements
for the degree of Doctor
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BALTIMORE, MD.

Botanical Contribution from the Johns Hopkins University, No. 94

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A PHYSIOLOGICAL STUDY OF BOTTLED AND
CULTURED IN APPLE AND WILLOW

THEODORE J. SWIFT

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A PHYSIOLOGICAL STUDY OF ROOTING AND CALLUSING IN APPLE AND WILLOW¹

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INTRODUCTION

In treatises on horticulture but two categories of cuttings are generally recognized—those of softwood and those of hardwood. However, hardwood cuttings may, in turn, be divided into two categories—those that have preformed small but unmistakable root primordia (already present when the cuttings are removed from the original plant) and those that do not possess such primordia. Where they are found, these preformed primordia seem to be simply very small roots or groups of cells that will produce roots upon further growth. Little is known as yet about them or the environmental influences that affect their formation, but they appear to be specific or varietal characteristics of certain plant forms (17).³ The present paper deals only with hardwood cuttings that have such preformed root primordia, apple cuttings (Springdale variety) and willow cuttings (*Salix alba* L.) being used.

Essentially, the rooting of cuttings with primordia consists simply in renewed growth, by which the small dormant roots elongate and push out beyond the bark. In the forms here studied the formation of these roots had occurred long before the cuttings were taken from the trees. These roots may be detected in the bark of 3-year-old and older branches of the Springdale apple, but they have not been observed in the younger wood. Apparently a certain degree of maturity is necessary before they begin to form.

The writer (21) has recently shown that such root primordia are formed by the cambium and apparently only at certain places where branch or leaf-trace parenchyma or primary or secondary rays cross the cambium. The primordia enlarge until the tips reach the outer surface of the bark or project a bit, and then they cease to enlarge and remain dormant until special conditions lead to renewed growth. They are apt to occur in small groups or in groups of 100 or even many more, as well as singly. The raised and modified areas of the bark in and beneath which they lie have long been called burrknots in

¹ Received for publication Mar. 13, 1928; issued July, 1929. This study represents one phase of nursery-stock investigations in the Bureau of Plant Industry of the U. S. Department of Agriculture. The work was done under the official direction of L. C. Corbett, Senior Horticulturist, in charge of the Office of Horticulture.

² For materials and equipment, for much help in planning and conducting the experiments, in interpreting the results, and in presenting them in this form, the writer wishes to express his appreciative thanks to Prof. Burton E. Livingston, Director of the Laboratory of Plant Physiology of the Johns Hopkins University. The writer has also been materially assisted from time to time by Dr. F. W. Haasis, of the same laboratory and of the U. S. Forest Service, and by Mrs. Mildred B. Swingle.

³ Reference is made by number (italic) to "Literature cited," p. 127.

England. The occurrence and formation of root primordia in certain varieties of apple, etc., that exhibit this peculiar phenomenon have been reported by the writer (17, 18, 19, 20).

The occurrence of preformed root primordia under the superficial bark layers in some willows has been demonstrated recently by Van der Lek (11) and by Priestley (14) and was mentioned by Trécul (22) in 1846. In the present studies these have not been given special attention further than to ascertain that they occurred on the willows used in the experiments and to make sure that the roots produced in these experiments were of the type here considered; that is, were actually produced by renewed growth of preformed, dormant roots. In willows, so far as known, there are no burrknots, for here the dormant roots occur only singly or in small groups and do not result in discernible enlargements. It appears, however, that *Salix alba* and the Springdale variety of apple are essentially alike in this particular, that both have preformed roots occurring in the bark of the older twigs. Vöchting (23) reported that root primordia were very much more numerous on stems of willow 2 years old and older than on the most recently formed stems.

ENVIRONMENTAL CONDITIONS NECESSARY FOR ROOTING AND CALLUSING OF CUTTINGS

The production of callus at the ends of cuttings or at lenticels often precedes or accompanies the "pushing" of roots. Callus is a form of tissue hypertrophy, sometimes resulting in large masses of white parenchyma, and it has been thought that there is some definite relation between callus production and root formation. It is still very generally held by propagators that new roots spring from the previously developed callus, in spite of the fact that Corbett (4) long ago pointed out that rooting and callusing are usually distinct processes. It seems probable, nevertheless, that roots may be induced to form from callus under some conditions.

Among the earliest analytical work on the rooting of cuttings were the important studies of Vöchting (23). According to his results with *Salix viminalis* L., gravitation had little or no influence on rooting, while light was found to retard it. His cuttings failed to root in the absence of oxygen.

Boehm (1, 2) stated that the rooting of willow cuttings was greatly retarded or practically prevented when the air about them had a pressure of 3 to 6 atmospheres.

Simon (15) found that callus growth in *Populus nigra* L. was most vigorous with humidities of 85 to 90 per cent. Grau (6) reported that an air humidity of 85 to 95 per cent, with a temperature of 25° C., produced abundant callus but few stem growing points in decapitated cuttings and grafts of tomato and nightshade. With the same temperature, but with air humidity of 60 to 75 per cent, little callus was produced, but many stem growing points were formed. With a maintained air humidity of 76 per cent, she found that the optimum temperature for callus growth was about 28° to 32°, while that for the production of stem growing points was about 22° to 28°.

Curtis (5) obtained results which indicate that rooting was stimulated by proper treatment of the cuttings of *Ligustrum ovalifolium* Hassk. with potassium permanganate, probably through an acceleration of the respiration processes.

Zimmerman (24) reported that willow cuttings failed to root when surrounded by gas containing 90 to 100 per cent of oxygen. The most active rooting in his experiments occurred with a gas mixture of oxygen and nitrogen containing 15 to 33.3 per cent of oxygen. Approximately "normal" growth occurred with 25 per cent of carbon dioxide in the gas about the cuttings, provided the oxygen content was between 25 and 33.3 per cent. According to Zimmerman's experimental results, the optimum temperature for rooting for Late Crawford peaches seemed to be at least as high as 24° C., while the optimum for *Plex verticillata* (L.) Gray seemed to be at least as high as 27°.

Regarding the influence of the hydrogen-ion concentration of the medium in which cuttings are rooted, apparently the only publications are those of Smith (16) and of Hitchcock and Zimmerman (7). The former worker found that *Coleus* cuttings rooted best in water with a pH value of 7.0 to 7.2, but there was some rooting throughout the range from pH 4.0 to pH 9.2. Inasmuch as acid peat with a pH value of about 4.6 is considered the best practical medium in which to root *Coleus* cuttings, she concluded:

These results indicate that the free admission of air is one of the most favourable aspects of the fibre and suggest that (for this plant at least) given equal supplies of oxygen, a reaction of the medium near neutrality would be preferable.

Hitchcock and Zimmerman (7) presented at the 1926 meeting of the American Society for Horticultural Science some results showing that certain acid-soil plants root better in peat than in sand and that certain others root better in sand than in peat. In the discussion of their paper it was brought out, however, that they had been unable to differentiate between the influences exerted by acidity of the medium and its capacity to supply oxygen and water. It may be that the acidity of the medium as such plays very little part in the rooting of plant cuttings within the ordinary ranges of horticultural experimentation and practice.

Knight (9) has recently reported that the production of callus on cuttings is favored by a higher soil moisture than is required for the production of abundant roots. He does not appear to have distinguished between the influence of high water content as such and its influence in limiting the oxygen supply.

GENERAL NATURE OF THE EXPERIMENTAL STUDIES

The experiments reported in this paper represent an attempt to obtain some additional knowledge, of a somewhat quantitative nature, about the temperature, water, and oxygen relations of root and callus development on cuttings of the Springdale variety of apple and of *Salix alba* L. This study is distinctly of a preliminary nature. It is intended to report the results of a particular group of experiments with particular kinds of cuttings and should not be used as a basis for generalizations about the behavior of plant cuttings in general or even of cuttings with preformed, dormant roots.

Though this paper deals only with the controlling external factors, it is necessary to emphasize the obvious but frequently neglected or slurred generalization that the formation of roots clearly depends not on internal conditions alone nor on external conditions alone but on both categories of influential conditions operating together.

The experimental data were nearly all obtained in the Laboratory of Plant Physiology of the Johns Hopkins University in 1926-27, although some preliminary explorational tests had been made earlier.

METHODS AND PROCEDURES

THE PLANT MATERIAL AND ITS PREPARATION

The apple cuttings used in these experiments were all obtained from one tree of the Springdale variety at the Arlington Experiment Farm, Rosslyn, Va. The willow cuttings were obtained from one tree of *Salix alba* on the grounds of the Johns Hopkins University. Other plant material used from time to time was obtained at the United States Plant Field Station, Glenn Dale, Md. The willow cuttings were usually put into the experiment chambers the day they were removed from the tree. The apple cuttings were put into closed suitcases when removed from the tree and remained there, without the addition of water, for one or two days at a temperature of about 19° C., until they were placed in the chambers in water. In the case of the apple cuttings, as in the case of all other material not placed either in the chambers or in water as soon as removed from the plant, a fresh cut was always made at each end of each cutting.

In each experiment great care was taken to insure like lots of material of each kind for all tests throughout the series. After being made, the cuttings were sorted into as many lots as would be needed, all lots being as nearly alike as possible. The apple cuttings were from wood 3 to 10 years old; most of the willow cuttings were from 2-year-old wood, but some 1-year-old and some 3-year-old wood was used. In most of the experiments 50 cuttings were placed in each chamber, the chambers being 3-liter bottles. In some cases all cuttings in any chamber were of one kind, either apple or willow, but there were generally two or more plant forms represented in each chamber.

No precautions were taken to secure or maintain sterile conditions in the chambers. At the higher temperatures, especially with low rates of oxygen supply, considerable amounts of mold developed, and some fermentation was generally evident at the end of the 10-day experiment period. There were no indications, however, that lack of sterile conditions played any appreciable part in the control of rooting and callusing in any test.

In some of the experiments the cuttings were subjected to a preliminary treatment with water, and in about the same number of experiments they received no preliminary treatment. In most cases the experiment period was either 7 or 10 days, counting from the time the cuttings were made and distributed into groups.

EXPERIMENTAL CONDITIONS

In most cases the cuttings of each test were brought under the special conditions for that test by means of experiment chambers, large-mouth bottles, generally with a capacity of 3 liters, but in some cases larger or smaller. These test bottles were closed with rubber stoppers, suitably provided with perforations in cases where flowing gas was employed, and each bottle contained some water at the bottom. In some of the earlier experiments the bases of the cuttings

rested on wet or merely moist cotton, supported on miniature perforated tables formed from blocks of paraffin, which rested on the bottom of the bottle in each case. It was found, however, that this arrangement was unsatisfactory, since the bases of the cuttings were more or less unequally supplied with water by the cotton. In later experiments the cuttings were in contact with gas only, each one being supported on a wire stilt, applied just before the cutting was placed in the chamber. The stilt consisted of a piece of iron "stove-pipe" wire wrapped once around the cutting near its base and projecting downward about 10 cm. beyond the lower end of the cutting. The free end of the wire rested on the bottom of the test bottle, and the cutting was supported so that no part of it came in contact with the water below.

The experimentation involved different treatments of the cuttings, with respect to (1) temperature, (2) water, and (3) oxygen conditions, which were more or less controlled artificially by special arrangements or procedures. The preliminary nature of the work and lack of time for further refinements made it undesirable or inexpedient to attempt precise controls, except in the case of temperature, which could be readily maintained within narrow limits of fluctuations. The influence of changing temperature was not studied, only continually maintained temperatures being employed.

TEMPERATURE

Most of the experimental results here reported were secured by means of a battery of seven dark chambers with automatically maintained temperatures. These temperature chambers have been described by Livingston and Fawcett (12). The seven chambers are vertical cylinders, each with a covered opening at the top, arranged in a row. Each chamber is surrounded by a water jacket, except above, where there is an insulating cover, and the water is mechanically stirred, which is also true of the air in the chamber. Each chamber is 33 cm. in diameter and 43 cm. deep. One end of the series is electrically heated and controlled; the other is electrically cooled and controlled. For each experiment the controls were set to give a series of seven different maintained temperatures. Temperature fluctuation in any chamber generally did not extend beyond 1° C. above and below the value shown, and fluctuation was usually much smaller. The largest fluctuations were of short duration; they were occasioned by necessary opening of the chambers and did not reach the cuttings in the test bottles.

The test bottles containing the cuttings were in the seven controlled chambers. When flowing gas was employed the tubes leading to and from the bottles entered the chamber through small holes in the top, alongside the removable cover.

Another arrangement for maintaining a single temperature on a very narrow temperature range was employed in some of the experiments. It consisted of a vertical, insulated, cylindrical tank of galvanized sheet iron, 70 cm. high and 50 cm. in diameter, filled with water and provided with a very positive mechanical stirrer operated by an electric motor, and also provided with an electric emersion heater, a mercury thermoregulator, and suitable relays. The test bottles containing the cuttings were suspended in the rapidly circulating water of the tank. No means was provided for cooling this tank,

and it was consequently used only for the maintenance of temperatures above the maximum one of the greenhouse room in which the apparatus was located.

A few experiments were carried out in the dark room of the laboratory, which provided a temperature generally about 19° C., with fluctuations between 18° and 22°.

MOISTURE

The moisture and conditions of the gas about the cuttings were those of approximate saturation for the temperature employed. As has been said, each test bottle had some water at the bottom. When flowing gas was employed the gas stream was first passed through water in a humidifying bottle, so that the gas entering the test bottle was practically saturated with water vapor. Upon entering, the gas bubbled through the standing water at the bottom of the bottle, and complete saturation was thus very nearly approached.

In some of the experiments the cuttings were inserted in the test bottles without any special preliminary treatment. Doubtless these cuttings had lost a little of their original water content, since they had been removed from the tree for a considerable period and had been brought into the warmer laboratory room from the cooler out of doors.

In some experiments the influence of preliminary wetting of the cuttings was studied. This preliminary treatment was applied just before the cuttings were placed in the test bottles, the aim being to increase more or less the water content of the cuttings, either throughout or near the cut ends or near the base alone. Several different preliminary treatments with water were employed, as shown just below and as summarized in the tabulated results. The time consumed in the preliminary treatment was, in every case, included in the 10-day period of the experiment.

(1) Cuttings were completely injected by being submerged in water, with the air above first nearly exhausted for 15 minutes and then allowed to reenter the injection chamber. They remained in the water 22 hours after atmospheric pressure had been resumed. (Experiment 8.)

(2) Cuttings were completely submerged in water for one hour without injection. (Experiment 14.)

(3) The bases of cuttings were submerged in water for 19, 22, 24, or 48 hours without injection. (Experiments 10, 9, 15, 16, respectively.)

(4) The bases only were injected (as in 1, above), and the bases remained under water for 19 hours after atmospheric pressure had been removed. (Experiments 11, 12.)

OXYGEN

In many of the experiments gas flowed through the test bottles throughout the experiment. The gas stream was passed through the bottle by means of suitable tubes borne by a 2-hole rubber stopper set in the bottle mouth. The rate of flow was maintained by means of constant pressure and properly adjusted orifice resistance, a cotton-kaolin orifice control like that devised by Hutchins (8) being used.

Constant pressure was maintained by means of a mercury pressure regulator with 4 cm. of mercury column and continuous escape of some gas. Several rates of gas flow were employed, approximately as follows:

300 c. c. per hour (experiments 1 to 5 and experiment 7, apple and willow).

50 c. c. per hour (experiment 6, apple and willow).

200 c. c. per hour (experiments 8 to 16, apple, and experiments 8 to 12, willow).

Flowing air was employed in experiments 2 to 4 and 8 to 12 (apple and willow) and in experiments 13 to 16 (apple). Flowing oxygen was employed in experiments 1 and 7 (apple and willow), and an artificially prepared mixture of oxygen and nitrogen was employed in experiments 5 and 6 (apple and willow), the mixture containing 5 per cent oxygen. Glass and lead tubing were used, with a minimum amount of rubber tubing for connections. When flowing air was used the requisite pressure gradient was maintained by means of an ordinary water aspirator pump attached to the laboratory water supply, the mercury pressure regulator and the orifice controls being suitably arranged. Air entered each test bottle from the maintained-temperature chamber in which the bottle stood, the air being thus at the proper temperature in each case, and it was discharged throughout the aspirator. When flowing oxygen or the artificial mixture of oxygen and nitrogen was used it was drawn from an ordinary commercial tank by means of a control valve, no pump being required.

The oxygen-nitrogen mixture was prepared in a commercial gas tank at high pressure, the proportions of the two gases being determined by their partial pressures in the mixture. The method used for preparing this mixture was that employed by Prof. E. K. Marshall, of the Johns Hopkins Medical School, who brought it to the writer's attention. In one case, for example, an empty tank was connected to a tank of nitrogen at an initial pressure of 1,800 pounds per square inch, the connection being made by means of a piece of suitable copper tubing 75 cm. long with a mechanical pressure gauge inserted in the middle and with a suitable coupling at each end. After the two tanks had been connected their valves were opened, and the pressure was allowed to become about the same in both. Both valves were then closed, and one tank was removed from the connecting tube and replaced by a tank of oxygen at a pressure of 1,200 pounds per square inch. After an hour, during which the nitrogen (cooled by this expansion) again assumed the temperature of the surroundings, the valve on the nitrogen tank was opened (the valve on the oxygen tank remaining closed), and the pressure of the nitrogen was read from the gauge; it was 910 pounds per square inch. Then the oxygen valve was opened, and oxygen was allowed to pass into the nitrogen in several installments till the gauge reading, with the oxygen valve again closed and after a suitable period for temperature recovery, was 958 pounds per square inch. Then both valves were closed, and the coupling tube was removed. The tank that had been originally empty now contained a mixture of nitrogen and oxygen, with partial pressures of 910 and 48 pounds per square inch, respectively; i. e., a mixture of approximately 95 per cent of oxygen. This method of preparing the gas mixture does not involve differential solubilities of the gases in a liquid, as when gasometers are used, and it

provides the mixture conveniently in a pressure tank, under pressure suitable for long runs of such experiments as are here considered. The limits of precision are set by the precision of the gauge readings.

Commercial nitrogen and oxygen such as were used in these studies are prepared by the distillation of liquid air and may be supposed to contain some impurities, which are limited to the constituents of air. The nitrogen contains a very little oxygen (perhaps 0.5 of 1 per cent), and the oxygen contains a corresponding amount of nitrogen. Carbon dioxide and argon are present in such small quantities that they may be ignored. Since tests of similar gases by other workers indicate no other impurities as likely to be present, no scrubbing arrangements were employed, and no analyses were made.

When only small quantities of gas mixtures were needed, other and less precise methods of mixing were employed. The ordinary procedure with a water gasometer was followed in some cases. In some of the preliminary experiments without maintained temperatures and without flowing gases, rubber bladders were used, the gas within the test bottle being thus maintained under slightly more than atmospheric pressure. With this arrangement, which was similar to that used by Free,⁴ only nitrogen, oxygen, or carbon dioxide was used with any given bottle and bladder, and mixtures were not attempted. In a few experiments a method described by Cannon (3) was employed. The oxygen of the air in an inverted test tube was practically all removed very rapidly by means of moist, bright steel wool in the closed end of the tube, the cuttings being initially placed in the tube and the lower end of the latter being closed with agar gel.

DURATION OF THE TESTS

It has been made clear by the work of Lehenbauer (10) on maize seedlings that the temperature graph for any form of plant growth may be expected to vary considerably with the length of the experiment period, especially with regard to the cardinal temperatures. If the period is very short, no growth is to be detected except at temperatures within or in the vicinity of the optimum range. This generalization probably applies to the study of the rooting and callusing of cuttings. A 10-day period was arbitrarily adopted for most of these tests, simply because this period proved long enough to give apparently satisfactory and consistent results. A 7-day period was used for some of the experiments with willow, especially those carried out in the single-chamber apparatus described above, maintained only at optimal or supra-optimal temperature.

OBSERVATIONS AND RECORDS

At the end of the 10-day period the cuttings were all removed from the test bottles and records were made of the amount of rooting and callusing that had occurred. The data thus secured were so averaged as to give a numerical index representing each form of activity at each temperature of an experiment. For callusing, observations were made on base and top callus separately.

⁴ FREE, E. E. THE OXYGEN REQUIREMENT OF PLANT ROOTS IN RELATION TO SOIL AERATION. (Diss. Johns Hopkins.) 1917. [Unpublished.] (See also reference 13, p. 128.)

EXPERIMENTS WITH APPLE CUTTINGS

The data from the experiments on rooting and callusing of apple cuttings in flowing gas and with maintained temperatures are presented in Table 1. For each temperature used (except the highest in each experiment) the tabulation shows an index value representing the "pushing" of roots and two values for the formation of callus, the first for the tops and the second for the bases of the cuttings, which were all placed with their basal ends downward. For the highest temperature value in each series no rooting or callusing values are given, since no activity of either sort was observed at these temperatures. Consequently, the temperature shown at the extreme right in each line of the table may be taken to represent either the temperature maximum for all three kinds of activity in the experiment in question or else a value somewhat higher than the maximum in each case.

The values given for pushing of roots represent in each instance the proportion of the cuttings used that showed more or less pushing of roots at the end of the 10-day period. These are calculated on the basis of 10; the value 10 indicates that every cutting showed some pushing of roots, the value 6 indicates that six-tenths of the cuttings showed this activity, and so on. These rooting values give no indication of the number of protruding roots observed or the lengths of the roots. If a cutting showed but a single slightly protruding root it was recorded as pushing, and the record was the same if there were several protruding roots, some of which were 1 cm. or more in length. It would doubtless have been more to the point if the number of root primordia had been determined for each cutting together with the number and length of the protruding roots at the time of observation, so that the index of pushing might have been based on the total number of primordia available instead of on the number of cuttings used, while the amount of growth after the roots emerged might have been shown quantitatively in terms of their elongation. In the case of the willow cuttings, however, the counting of primordia would not have been possible for these are not visible.

The amount of callus developed at the top and at the base of the cuttings during the 10-day period is shown in the table by relative numerical values between 0 (no callus observed) and 10 (the maximum amount of callus observed in this entire study). The amount of callus formed per cutting was not measured precisely, and these data are based on a somewhat rough scoring method that was developed during the progress of the studies. The score value 10 indicates that the region of the cutting considered (top or base) was about half covered with thick, apparently vigorous callus.

TABLE 1.—Numerical values for pushing of roots and for growth of callus of apple cuttings

Experiment No.	Date started	Conditions	Temperature		Roots ^a	Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a		Callus ^b		Temperature	Roots ^a	
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Besides the experiments with both flowing gas and seven different maintained temperatures, several experiments were carried out with other treatments. The results of some of these additional experiments are given hereinafter in Tables 3, 4, and 5. Table 3 presents the numerical results of experiments 17, 18, and 19, with maintained temperatures but without flowing gas. These results are arranged like those in Table 1 except that values for lenticel callus-formation are here included. The latter values are based on a scale of 10 like those for top and base callus formation. Table 4 gives the rooting score values from experiments 20 to 23, with different treatments, conducted in a series of four chambers and in the laboratory dark room. In two other dark-room experiments, sealed chambers of different capacities were used, the chambers of experiment 24 being filled with air and those of experiment 25 being filled with oxygen at the start. The numerical values for rooting and callusing from these experiments are given in Table 5, on a scale of 10, just as in Tables 1, 2, and 3.

The data given in the tables will be generally discussed with reference to temperature graphs that have been drawn to represent each series.

ROOTING OF APPLE CUTTINGS

EXPERIMENTS WITH FLOWING GAS AND SEVEN DIFFERENT MAINTAINED TEMPERATURES

This section deals with the data given in Table 1 and with the corresponding graphs. (In all the charts, figs. 1 to 17, the numbers on the ordinate indicate relative degrees of rooting or callusing on a scale of 10.) Experiment 1, started November 8, 1926, gave what appears to be the usual form of temperature curve for growth, with minimum about 8° C. or higher, maximum about 39° or lower, and optimum about 28°. (Fig. 1.) These cuttings were well supplied with water-saturated oxygen, and the bases were not covered by water, for the cotton on which they rested was not nearly saturated. It is possible, however, that some water was absorbed from the damp cotton. Experiment 2 (fig. 1), started November 23, 1926, was like experiment 1, except that air instead of oxygen was continually supplied. The temperature maximum here is indicated as below 41°, and it may have been about 39° or lower, as in experiment 1. The optimum for experiment 2 is not so clearly shown as that for experiment 1, but it may have been about the same temperature as that for experiment 1 or somewhat higher. Experiment 2 differed from experiment 1, however, with respect to the minimum, which appears to have been above 18°, perhaps even about 20°. No explanation of this difference is apparent, for it seems hardly probable that the lower oxygen supply of the second series could have retarded

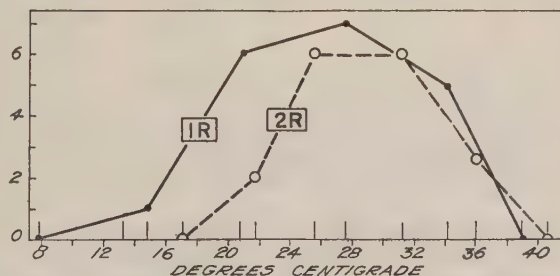


FIGURE 1.—Rooting of apple cuttings in closed chambers: 1R, in flowing oxygen; 2R, in flowing air

root pushing for the lower temperatures without showing a still more marked retardation for the higher ones. The dates are nearly the same for the two experiments, and seasonal differences can hardly be considered as explaining the observed differences.

Experiments 3 and 4 (fig. 2) were planned to compare the behavior of cuttings with bases surrounded by wet cotton with that of other cuttings with bases in water-saturated air, both series being otherwise alike, continually supplied with flowing air. It appears that the wet cotton wrappings at the bases of the cuttings retarded the pushing of the roots (fig. 2, 3R), for no roots emerged except at temperatures about 30° and 35° C.; furthermore, emerging roots were here confined to the upper half of the cuttings, and the amount of root growth was

noted as very slight. The minimum in this case seems to have been above 26°, the maximum below 40°, and the optimum perhaps about 34° or 35°. In experiment 4, however, with no water films about the bases, the growth curve indicated is of the usual form, with minimum about 18° and maximum below 40°, while the optimum is indicated in this case as about 30°, though it may have been lower. This graph for experiment 4 is similar to that for experiment 2, which had similar conditions. It is remarkable that the more plentiful oxygen supply of experiment 1 seems to have accelerated the pushing of roots at the lower temperatures, giving a much lower apparent minimum than that shown for experiments 2 and 4. If it be supposed that the wet cotton of experiment 3 acted to retard the access of oxygen to the cuttings, then it may be significant that this wet-cotton treatment appears, when compared with experiment 1, to have produced a retardation similar to that of experiments 2 and 4 but still more pronounced.

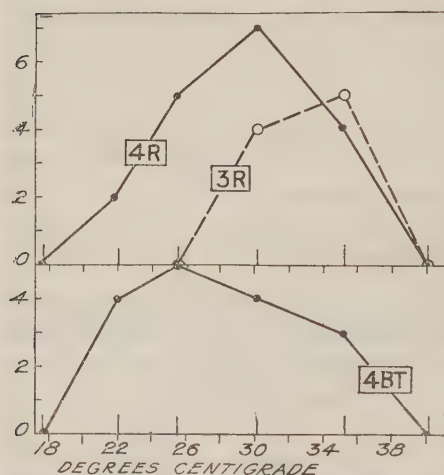


FIGURE 2.—Rooting (3R and 4R) and base-callus and top-callus formation (4BT) of apple cuttings in closed chambers with flowing air; 3R, bases of the cuttings resting on wet cotton; 4R, bases resting on wire stilts

minimum than that shown for experiments 2 and 4. If it be supposed that the wet cotton of experiment 3 acted to retard the access of oxygen to the cuttings, then it may be significant that this wet-cotton treatment appears, when compared with experiment 1, to have produced a retardation similar to that of experiments 2 and 4 but still more pronounced.

In Figure 3 are presented this comparison between the data for experiments 2 and 4 (considered as alike) on the one hand and experiment 13 on the other, which indicates a pronounced difference that may be related to the season of the year at which the experiments in question were begun. The lower graph of the upper part (2, 4R) represents the average of experiments 2 and 4 (November 23 and December 7), and the upper graph (13R) represents experiment 13 (February 26). With respect to external conditions these two graphs represent no considerable differences, and the marked difference shown seems to have been due to changes that had occurred in the twigs themselves between November and March. It appears that the optimum temperature was definitely lower (about 24° C.) and that the graph was much flatter at the top for the later experiment, and also that the minimum was much lower (probably below 10°

and perhaps as low as 9°). The maximum temperature appears to be about the same for both seasons (about 40°).

Experiments 5 and 6 (fig. 4) were characterized by a low oxygen content in the flowing gas (5 per cent of oxygen and 95 per cent of nitrogen) and were planned to bring out a comparison between rapid and slow flow, the rate of flow for the gas mixture being only one-sixth as rapid for experiment 6 as for experiment 5. The oxygen supply for experiment 5 was lower than that for experiments 2, 3, and 4 (with flowing air) and much lower than that for experiment 1 (with flowing oxygen).

The graphs of experiments 5 and 6 agree very closely. Apparently these two sets of conditions were too nearly alike to bring out significant differences in the pushing of the roots. Only with respect to the maximum temperature do these graphs disagree, and it is to be noted that, although no pushing was observed at about 33°C . in experiment 6, yet the amount of pushing for this temperature in the other experiment was very small. If the two series are considered together, the minimum may be estimated as above 14° , the maximum as about 34° , and the optimum as about 24° to 26° . Apparently the low concentration of oxygen

here acted chiefly to reduce the index values for the supra-optimal temperatures and to shift the maximum toward the left.

Experiment 7 (fig. 3, lower part; fig. 5, upper part) resembled experiment 1, but the cuttings of the later series were entirely surrounded by moist gas (being supported on wire stilts) while those of the earlier series rested on damp cotton. The two experiments agree in respect to the cardinal temperatures (about 8° , 28° , 39°C .), but the values for rooting are higher for experiment 1 in the intermediate suboptimal temperature range. It is not easy to relate this difference to the seasonal difference between these two series, for it is in the opposite direction from that shown between experiments 2 and 4 on the one hand and experiment 13 on the other. Experiments 2, 4, and 13 were with flowing air, while experiments 1 and 7 were with flowing oxygen, but this circumstance can hardly give a clue to an explanation of the observed difference between the results of the two

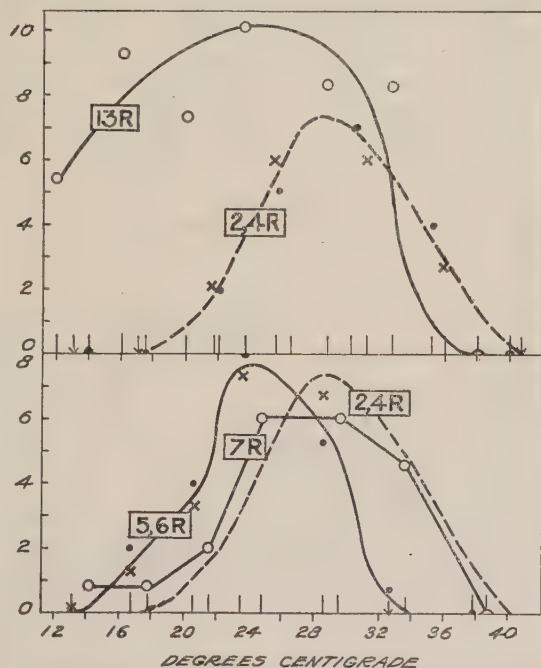


FIGURE 3.—Upper portion: Seasonal differences in the rooting of apple cuttings in closed chambers with flowing air. Lower portion: Rooting of apple cuttings in closed chambers with different flowing gases, experiments 2 and 4, air; 5 and 6, 5 per cent oxygen in nitrogen; 7, oxygen

series. It is suggested that the difference between the graphs for experiments 1 and 7 may be related to water supply, for the cuttings of the earlier series rested on damp cotton, whereas those of the later series were entirely surrounded by moist gas, as has been noted. However, the remarkable similarity of the graphs for experiments 2 and 4

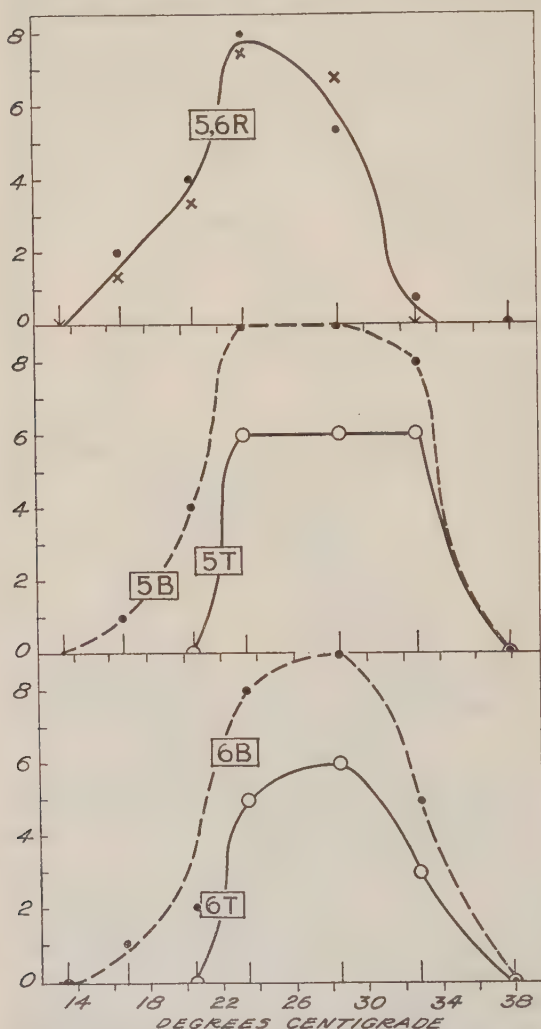


FIGURE 4.—Rooting (5, 6 R), top-callus formation (5T, 6T), and base-callus formation (5B, 6B) of apple cuttings in closed chambers in a flowing-gas mixture of 5 per cent oxygen in nitrogen. Experiment 5, 300 c. c. gas per hour; experiment 6, 50 c. c. per hour

render such an explanation at least doubtful, unless the difference between flowing air and flowing oxygen is considered as influential in this respect, for the cuttings of experiment 2 had the same moisture treatment as those of experiment 1, and the cuttings of experiment 4 had the same moisture treatment as those of experiment 7. The difference between the graphs for experiments 1 and 7 were related to unknown conditions.

Experiments 8 and 9 (fig. 6, upper part) were alike except for preliminary water treatment, both having flowing air. For experiment 8 the cuttings had been first injected and then completely submerged in water for 22 hours. For experiment 9 they had not been injected, and only the bases had been submerged during a soaking period of 22 hours. It is clear that the more abundant water treatment in experiment 8 greatly retarded the pushing of roots and completely inhibited it for temperatures above about 24° C.

The graph for experiment 9 has a very flat-topped form, indicating a broad range of approximately optimal temperatures. An optimum temperature in this case can not be estimated; it is represented by a range from about 14° to 34° C. No minimum temperature was

reached, but it must be estimated as probably as low as 8° or 9° , and the maximum was about 39° .

The exceptionally broad optimal range shown by the values from experiment 9 suggests that some special preliminary water treatment may be regarded as beneficial, although it must not be too extensive. Experiments 10, 11, and 12 (fig. 7, upper part), the last two being duplicates, gave graphs essentially much like that for experiment 9 and appear to support this suggestion. All had preliminary water treatment, and the optimal range seems to have extended from about 16° to about 29° C., if the results of the three tests are combined. Soaking alone (experiment 10) gave the broader optimal range, while an injection at the beginning of soaking seems to have narrowed this range somewhat at both ends. Experiments 9, 10, 11, and 12 appear to show that rooting in February was not much influenced by temperature differences between about 16° and 33° when some extra water was added to the tissues by preliminary water treatment. But the data from experiment 8 indicate, as has been said, that such preliminary water treatment may be excessive, resulting in a pronounced retardation and even in a complete inhibition of the pushing of roots. It may be that the internal water supply was somewhat inadequate in the cuttings used at that time, unless preliminary water treatment had been given. The retarding action of excessive preliminary treatment with water may have been due to a water plugging of air spaces in the tissues of the cuttings, this plugging perhaps operating to retard the supply of oxygen to the root primordia.

The growth values for the optimal temperatures were very high (9) in these experiments, as well as in experiment 9. As to the indications regarding the minimum and maximum temperatures, experiments 9, 10, 11, and 12 suggest a low minimum, hardly above 8° or 9° C., while they agree very well in indicating a maximum of about 38° to 39° .

Experiments 13, 14, 15, and 16 (figs. 8 and 9) were all carried out at the same time, beginning February 26, 1927, and all had the same gas treatment (flowing air). They were planned to throw more light on the relation of root pushing to preliminary water treatment. The cuttings of experiment 13 were without such treatment, while those of the remaining tests received different water treatments. If the graph of experiment 13 is employed as a standard for comparison, the graphs of Figure 8 indicate that the optimal range was markedly widened by completely soaking the cuttings one hour at the start of the test. This range and the form of the temperature graph were



FIGURE 5.—Rooting (7R), top-callus formation (7T), and base-callus formation (7B) of apple cuttings in closed chambers with flowing oxygen

not markedly altered from those shown for experiment 13 by a preliminary soaking of the bases of the cuttings for 24 hours (experiment 15), and the range was much shortened and the graph was also much depressed (for the supra-optimal temperatures) when the bases were soaked for a preliminary period of 48 hours (experiment 16).

It appears that these cuttings, made February 26, were greatly improved with regard to rooting at suboptimal and supra-optimal temperatures (with flowing, water-saturated air) if they had been given the preliminary water treatment of experiment 14, that they were not seriously affected by the treatment of experiment 15, but that the water treatment of experiment 16 was markedly excessive (with consequent retardation) for temperatures above about 26° C. These tests seem to strengthen the suggestion derived from experiments 8 to 12. When the soaking at the bases of the cuttings was limited to a period of 24 hours, it appears that the favorable influence of preliminary soaking (perhaps due to a resulting increased water content of the cuttings) nearly offset a concomitant unfavorable influence (perhaps related to decreased oxygen supply), but that when soaking was prolonged to 48 hours the unfavorable influence greatly predominated, especially for supra-optimal temperatures.

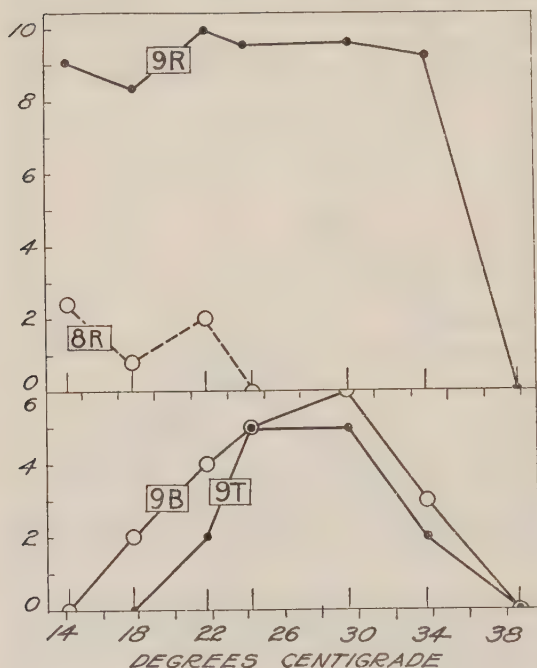


FIGURE 6.—Rooting (8R, 9R), top-callus formation (9T), and base-callus formation (9B) of apple cuttings in closed chambers with flowing air. The cuttings of experiment 8 were injected with water and remained completely immersed for 22 hours, while those of experiment 9 were soaked in water (bases only) for same length of time

10, 11, and 12, in showing very high values (10) for the indices of rooting activity. They also agree in indicating low minimal temperature values (below 8° C. for experiment 14) and in indicating a temperature maximum about 38° (except for experiment 16, with excessive preliminary water treatment, for which the temperature maximum appears to have been in the vicinity of 33°).

In addition to the numerical data taken from line 15 of Table 1, and represented by the upper line of the upper portion of Figure 10, the lower line (14r) of the same figure shows an estimate of the actual temperature curve of root pushing based upon additional observations made at the completion of experiment 14. It was noted that the largest number of roots per cutting and the greatest growth in length

occurred at about 23.7° C. and at about 28.7° . At these temperatures almost every available primordium was observed to be pushing, and roots 2 or 3 cm. in length were evident in many cases. The same amount of root growth was evident for 23.7° and for 28.7° , but roots were fewer and shorter at a temperature of 32.8° and at temperatures of 20.3° and lower; however, as has been stated, some root growth was observed on nearly every cutting. Similar qualitative estimates on number of roots per cutting and average root length were made at the completion of experiment 9 (bases soaked 22 hours) and experiments 10, 11, and 12 (bases soaked 19 hours). In all of these five experiments it was observed that the amount of growth in length as well as the proportion of available primordia that had pushed seemed to be the same at about 24° and about 29° .

Although no cuttings were tested at temperatures below 12° C. in experiment 14, the graph for this experiment (fig. 10, 14r) is extended to the zero line at 9° , for while every cutting subjected to a temperature of about 12° showed some root activity, such activity was so slight as to indicate that the zero point for discernible root growth within 10 days was not far from 9° .

It is possible from these 16 experiments to assign 41° C. as the upper temperature limit at and

beyond which no root pushing took place, but no definite minimum is apparent. Although the number of roots per cutting was small and the growth in length was very slight, nevertheless a very slow growth rather than the complete stopping of growth seemed to be indicated for the lowest temperatures tested. In fact, subsequent results showed that the absolute minimum temperature for pushing of roots was below 4.5° . Branches from 1 to 2.5 meters long were cut from the tree on February 23 and were placed with their bases in water, and when these were examined May 14 they showed a very slight amount of pushing by almost every avail-

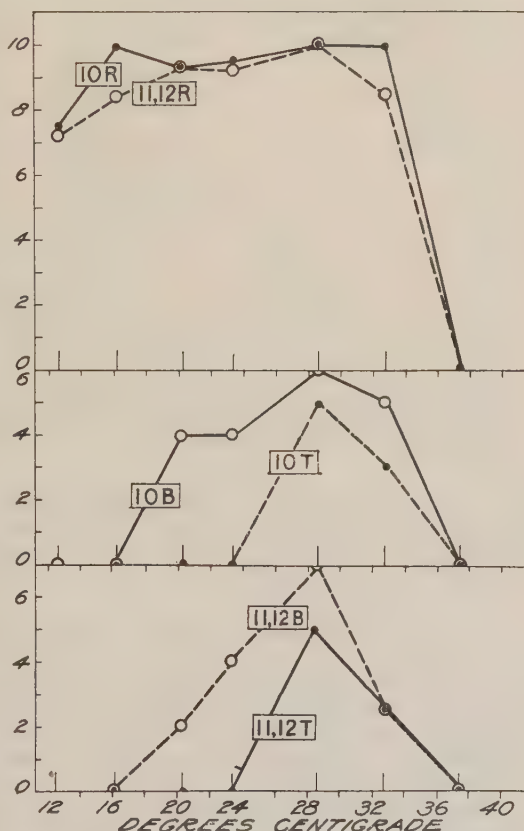


FIGURE 7.—Rooting (IOR, 11, 12R), top-callus formation (IOB, 11, 12T), and base-callus formation (IOB, 11, 12B) of apple cuttings in closed chambers with flowing air. Experiment 10, bases of cuttings soaked 19 hours; experiments 11 and 12, bases injected with water and soaked 19 hours

able primordium except those actually in the water. Nevertheless, growth of buds seemed to have been entirely stopped at this temperature (4.5°).

A comparison of the graph of experiment 13 with the corresponding one of experiments 2 and 4 (see fig. 3) indicates, as has been noted,

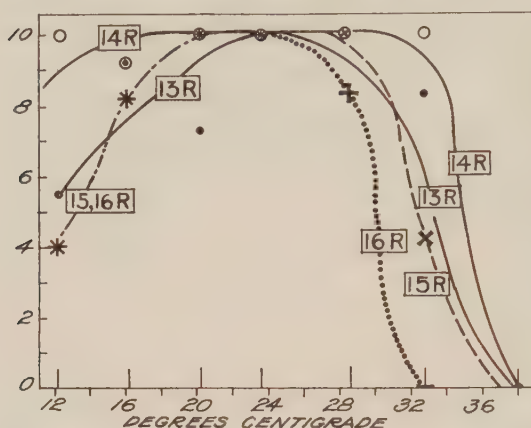


FIGURE 8.—Rooting of apple cuttings in closed chambers with flowing air and with different water treatments: Experiment 13, no water treatment; experiment 14, material completely soaked 1 hour; experiment 15, bases soaked 24 hours; experiment 16, bases soaked 48 hours

that the results of the later series differed from those of the earlier ones by showing a much greater rooting activity for the maintained temperatures below about 30°C . This apparent seasonal relation (perhaps due to differences in what may be termed the physiological maturity of the root primordia and their surrounding tissues) seems to have resulted (1) in shifting the later optimal temperature (experiment 13) downward on the temperature scale from about

30° to about 24° , (2) in shifting the minimum temperature in a similar manner (from about 18° to 8° or 9° , or lower), and (3) in markedly raising the index values for all temperatures below the supra-optimal temperatures.

SUMMARY OF RESULTS OF EXPERIMENTS 1 TO 16

The main numerical results of the several series are brought together for convenient comparison in Table 2, which is arranged to bring out the relations with regard to the degree of rooting for maintained temperatures near the optimum and with regard to the water treatment.

The later series, beginning on or after February 3, all gave very high values for the index of rooting (9 or 10) for the optimum temperature, with the single exception of experiment 8, the cuttings of which apparently had had excessive preliminary treatment with water. None of the earlier series gave index values higher than 8, no matter what the treatment. On the other hand, none of the earlier series gave these values lower than 6, except when the bases of the cuttings rested on wet cotton (experiment 3, with index value of 5). While this evidence is not very pronounced, it suggests that these cuttings had a somewhat greater tendency to root, under the range of conditions tested, in the latter part of the season than they had in the earlier part.

TABLE 2.—Summary of results of experiments 1 to 16 in rooting and callusing of apple cuttings

Experi- ment number	Date started	Flowing gas	Water conditions	Data for rooting			Data for top callus				Data for base callus				
				Approximate cardinal temperatures			Index for opti- mum temper- ature	Approximate cardinal temperatures			Index for opti- mum temper- ature	Approximate cardinal temperatures			Index for opti- mum temper- ature
				Mini- mum	Opti- mum	Maxi- mum		Mini- mum	Opti- mum	Maxi- mum		Mini- mum	Opti- mum	Maxi- mum	
1	1926 Nov. 8	Water saturated O ₂	Bases on damp cotton.....	° C. 8	° C. 28	° C. 39	7	° C. (a)	° C. (a)	° C. (a)	(a)	° C. (a)	° C. (a)	° C. (a)	(a)
7	1927 Jan. 21	do.....	Cuttings not in contact with liquid water.	8(?)	28	39	6	22	30	34	2	18	30	38	3
5	Jan. 5	Water-saturated mixture: 5 per cent O ₂	do.....	14	24	34	8	20	24-35	38	6	14	24-29	38	9
6	do.....	96 per cent N ₂	do.....	14	24	34	7	20	29	38	6	14	29	38	9
4	1926 Dec. 7	Water-saturated air.....	do.....	18	30	40	7	18	26	40	5	18	26	40	5
13	1927 Feb. 26	do.....	do.....	9(?)	24	38	10	16	24-29	38	6	12	29	38	9
2	1926 Nov. 23	do.....	Bases on damp cotton.....	18	28	40	6	(a)	(a)	(a)	(a)	(a)	(a)	(a)	(a)
14	1927 Feb. 26	do.....	Completely soaked 1 hour.....	8(?)	14-32	38	10	16	29	33	8	16	29	38	9
10	Feb. 15	do.....	Bases soaked 19 hours.....	8(?)	16-33	38	10	23	29	38	5	16	29	38	6
9	Feb. 3	do.....	Bases soaked 22 hours.....	8(?)	14-34	39	10	18	24-30	39	5	14	30	39	6
15	Feb. 26	do.....	Bases soaked 24 hours.....	9(?)	20-29	38	10	16	29	33	5	12	16-24	33	3
16	do.....	do.....	Bases soaked 48 hours.....	9(?)	21-24	33	10	24	29	33	5	24	29	33	2
11, 12	Feb. 15	do.....	Bases injected and soaked 19 hours.	9(?)	20-29	38	10	23	29	38	5	16	29	38	7
3	1926 Dec. 7	do.....	Bases on wet cotton.....	26	35	40	5	26	30	35	2	(a)	(b)	(b)	(b)
8	1927 Feb. 3	do.....	Injected and completely soaked 22 hours.	8(?)	14-22	24	2	(b)	(b)	(b)	(b)	(b)	(b)	(b)	(b)

a No observation.

b No callus.

It is also apparent from Table 2 that preliminary water treatment tended to lower the temperature minimum and to extend the optimal temperature range. Although it can not be definitely stated from these preliminary data, it seems to be indicated that the temperature minimum and the optimal temperature range were somehow partially determined by the water relations, being also influenced, perhaps, by internal conditions that were innate in the cuttings themselves at the beginning of a test, conditions connoted by the vague expression "maturity of tissues." The relative intensity of rooting, mentioned

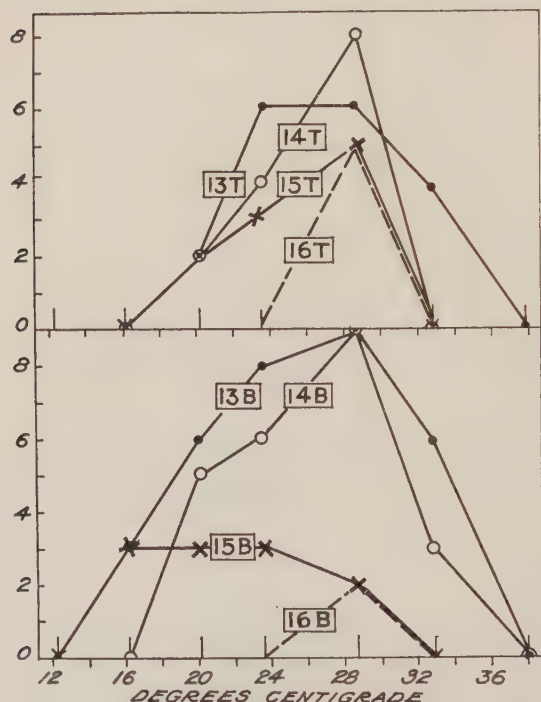


FIGURE 9.—Top-callus formation (13T, 14T, 15T, and 16T) and base-callus formation (13B, 14B, 15B, and 16B) of apple cuttings in closed chambers with flowing air and with different water treatments: Experiment 13, no water treatment; experiment 14, material completely soaked 1 hour; experiment 15, bases soaked 24 hours; experiment 16, bases soaked 48 hours

curve of rooting at optimal and supra-optimal temperatures without materially affecting the curve at sub-optimal temperatures. Further effects of low supplies of oxygen, especially at the higher temperatures, were apparent in experiments 8, 10, 15, and 16, where probably a considerable part of the residual air of the cuttings had been replaced by water. In such cases it seems that the oxygen supply and the temperature were very closely related; the higher the temperature the greater the supply of oxygen required for optimum growth.

As is shown by the graphs, in none of the experiments in which gas was flowing through the chambers did it seem to be indicated that large amounts or high pressures of oxygen had exerted any retardation upon the rooting of the apple cuttings. On the other

just above, seems to have been influenced by maturity. The optimum temperature for rooting can not be fixed from the data at hand, though it is probably safe to guess that it lay generally in the vicinity of 26° to 28° C., except in some cases where special treatment had been applied to the cuttings. With the exception of excessively wet cuttings and those in experiments 5 and 6, which were subjected to low concentrations of oxygen, it seems that the maximum temperature was about 39°, and this did not appear to alter with maturity or with the season of the year.

Independently of any effect brought about by water content, experiments 5 and 6 show that low supplies of oxygen may change the

hand, the use of flowing oxygen did not seem to show any particular advantage over flowing air in the rooting of these cuttings. Thus from these experiments the use of partial pressures of oxygen higher than that in air with a total pressure of 1 atmosphere does not seem to be desirable.

CALLUSING OF APPLE CUTTINGS

In spite of the conclusion that callusing is not as important in the rooting of cuttings as it is commonly considered, studies regarding callus formation are likely to prove of importance, especially for such light as they may shed upon the callusing of grafts. In practical horticulture, at least two very important desiderata are here suggested: (1) The production of sufficient callus to make a mechanically and physiologically satisfactory union, and, (2) the prevention of such callus overgrowths at the union as are frequently confused with the galls brought about by the crown-gall organism, *Bacterium tumefaciens* S. and T.

Records of callus formation were not made for the intermediate temperatures of experiments 1 and 2. In experiment 3 (Table 1), with flowing air and bases of cuttings on wet cotton, there was practically no callus formation either at bases or tops, and the very slight callusing observed occurred at the tops of 3 of the 10 cuttings with maintained temperature of 30.3° C. In experiment 4, however (Table 1), which was like experiment 3 except that wire stilts were used instead of wet cotton, there was pronounced callusing at intermediate temperatures, and the graph of callus formation in this series (fig. 2, 4BT) has the form of growth-temperature curves in general. No differences between top callus and base callus were observed in this case, although such differences were shown for all the subsequent experiments with apple cuttings, as will be seen from the tabulated data. The graph indicates an optimum temperature of about 26° C. and minimum and maximum temperatures of about 18° and 40°, respectively. On comparing this callus graph with the corresponding graph for rooting (fig. 2, 4R), it is seen that the two are

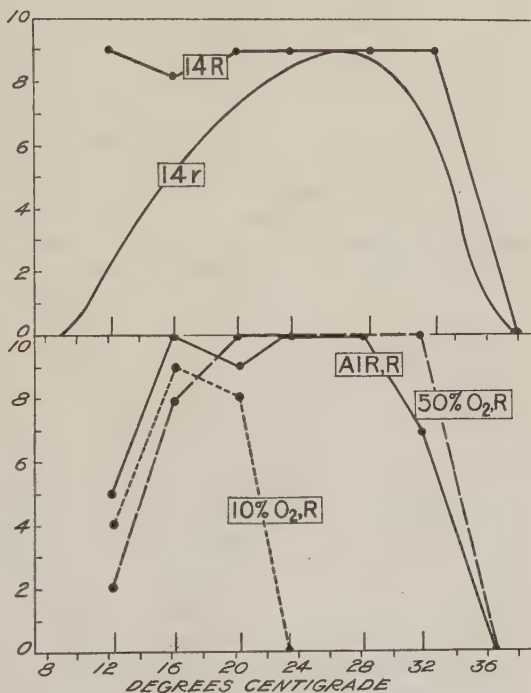


FIGURE 10.—Upper portion: Rooting of apple cuttings in closed chambers with flowing air, the cuttings having been preliminarily soaked one hour; 14R, the numerical results of experiment 14; 14r, estimated curve of actual rooting, based on experiment 14 and additional observations. Lower portion: Rooting of apple cuttings in sealed chambers filled with air, equal parts oxygen and nitrogen, or with 10 per cent oxygen in nitrogen, at the start of the test, no more interchange of gas taking place

very similar in form as well as in height, although it appears that the optimum temperature was somewhat higher for rooting than for callusing; but the rooting optimum shown for this experiment is unusually high for such optima.

That callus was plentifully formed at the intermediate temperatures in experiment 4 but was practically inhibited at all temperatures in experiment 3 suggests that this apparent inhibition may have been due to excess of water about the cutting bases in the latter case. An extra experiment was carried out with but a single temperature (27° C.), which showed that callus formation might be secured in spite of the presence of wet cotton about the bases of the cuttings. In this case there were four treatments, all with wet cotton at the bases but differing with respect to the rate of air movement through the bottles. The most rapid air flow was like that of experiment 3 (300 c. c. per hour), and the other rates were progressively lower (50, 20, and 10 c. c. per hour, respectively).

As in experiment 3, no base callus was observed with any of these four treatments, and top callus was extremely meager and practically negligible for the higher rates of air flow. However, it seems significant that the two tests with slowest air flow (20 and 10 c. c. per hour) showed top callus unquestionably more in evidence than it was in the cases where air flow was more rapid. Seven out of 10 cuttings showed small amounts of top callus in each of these cases. This extra experiment suggests that the retardation of top callus indicated by the results of experiment 3 might be offset to some extent with slower air change than was employed in experiment 3. Such a relation between callusing and rate of air flow might conceivably be due to differences in the evaporating power of the air corresponding to the different rates of flow, but this seems unlikely, since the air was practically saturated with water vapor in all these tests. It is suggested that the differences in top-callus formation here in question may have been due to differences in the oxygen supply corresponding to the differences in rate of flow. According to such a supposition, in support of which additional evidence will be given below, the extreme retardation in top-callus formation shown by the results of experiment 3 and by those of these two special tests with relatively high rates of air flow, may be regarded as due to too-rapid supply of oxygen. When air was supplied at a very slow rate (20 or 10 c. c. per hour), top callus developed much better than when the rate was more rapid (50 or 300 c. c. per hour). Other *a priori* considerations would require attention, however, if the interesting question thus raised were to be seriously studied. For an example of such considerations, products given off by the cuttings or by microorganisms might be more rapidly removed from the bottles with more rapid air renewal than from those with slower flow.

Perhaps the following statement may express the indications from experiments 3 and 4 and from the extra series: It appears that an excess of water about the bases of the cuttings inhibited callus formation at the bases and greatly retarded it at the tops, even for maintained temperatures in the optimal range, but that this retardation tended to disappear when the oxygen supply was much lower than in experiment 3.

In experiments 5 and 6 (Table 1) callusing was very active at both base and top for intermediate temperatures. It was about 33 per

cent less active at top than at base in the optimal temperature range. Graphs for the callus values from these two experiments are shown in Figure 4. In these cases the flowing gas was a mixture of oxygen (5 per cent) and nitrogen (95 per cent), and the rate of flow was more rapid (300 c. c. per hour) in experiment 5 than in experiment 6 (50 c. c. per hour). The minimum and maximum temperatures appear to have been about 13° and 38° for top callus, and the optimum with the slower flow of gas is indicated as about 28° for both base and top. In their optimal ranges the graphs for rapid gas flow are much flatter across the top than are the graphs for slow flow. Of the two rates of oxygen supply here tested, the more rapid rate produced more callus for sub-optimal and supra-optimal temperatures than did the slower rate, and it is therefore possible that a still more rapid rate of flow might have given an optimal range extending somewhat farther to the right, since the optimum rate of oxygen supply should be greater for the upper portion of the temperature range dealt with than for the lower portion. It is remarkable that a definite temperature optimum is not shown by either graph of experiment 5, although optima (about 28°) are clearly indicated by both graphs of experiment 6. From the results for 32.8°, it seems as if callusing at both top and base may be retarded by insufficient oxygen supply.

A comparison of callusing with rooting in experiments 5 and 6 indicates that the optimum and maximum temperatures were lower for rooting than for callusing. It is suggested that the minimum rate of oxygen supply necessary for rooting is significantly higher than the corresponding minimum rate for callusing.

Experiment 7 (fig. 5) with flowing oxygen gave more base callus than top callus, but callus formation was markedly retarded at all temperatures tested, as compared with the results of experiments 4, 5, and 6. From experiment 7 the highest score value for callusing is 3 (temperature about 30°) while the corresponding best callus score for experiment 4 is 5 (temperature about 26°) and for both experiments 5 and 6 it is 9 (temperature about 23° to 29°). A reason for this may be that in experiment 7 the oxygen supply was too great for excellent callusing at any temperature employed. It seems to be significant that the high oxygen supply of this test gave less callusing about the optimum temperature than was shown in any of the other experiments. It appears as though, for optimal temperatures and adequate moisture conditions, callus formation may differ from rooting by not requiring so much oxygen, and also by requiring an actually lower supply of oxygen. In other words, the lower limit of the range of oxygen supply that permits best growth may be lower for callusing than for rooting, and the extent of this range (from its lower to its upper limit) may be shorter for callusing than for rooting.

Experiments 8 and 9 in which flowing air was used were alike except for preliminary water treatment. The cuttings of experiment 8 had been injected with water and then completely submerged for 22 hours, whereas those of experiment 9 had not been injected, and only their bases had been submerged for the 22-hour period. No callusing was observed in experiment 8, but good callus formation occurred at the optimal temperatures in experiment 9 (fig. 6), which gave a maximum callus score of 6 (temperature about 29°). From these two comparative tests it appears that excessive preliminary water treatment greatly retarded rooting and completely inhibited callusing.

The graph of callusing for experiment 9 (fig. 6) is in general typical, showing a marked tendency for base callus to surpass top callus. The minimum temperature is shown as somewhat lower for base callus (about 14°) than for top callus (about 18°), the maximum temperature is the same for both base and top callus (about 39°), and the optimal region extends from about 25° to about 30° . The treatment of experiment 9 seems to have been very good for both rooting and callusing, but not so good for callusing as were the treatments of experiments 5 and 6.

Experiments 10, 11, and 12, the last two being duplicates and all three having flowing air, constitute another test of the influence of preliminary water treatment along the subsequent gas treatment of flowing air. The cuttings of experiment 10 had their bases soaked 19 hours, while those of experiments 11 and 12 had their bases first injected with water and then soaked 19 hours. The graphs of these data are shown in Figure 7. It is not clear that any significant difference was brought out. As in the case of rooting in the same experiments, both treatments appear to have been good. The usual difference between top and base callus is brought out for both of these water treatments, and the temperature optimum for callusing is shown as about 29° for both treatments.

Experiments 13, 14, 15, and 16 were planned to obtain further evidence on the effect of preliminary water treatment on callusing as well as on rooting. The data for callus formation are presented graphically in Figure 9, and may be compared with those for rooting shown in Figure 8. The four treatments, all with flowing air, were as follows: Without preliminary water treatment (experiment 13); cuttings completely submerged in water for 1 hour (experiment 14); bases soaked in water for 24 hours (experiment 15); bases soaked in water for 48 hours (experiment 16). Although the evidence is not as clear as it might have been had this series been repeated several times so that averages could be secured, yet a study of Figure 9 indicates that in general both base and top callus were markedly decreased by soaking and that the retarding effect upon callusing seemed to be at least roughly proportional to the amount of soaking.

Excessive preliminary water treatment is again shown to diminish callus formation at each temperature within the total temperature range, without shifting of the optimum temperature. For rooting (fig. 8) the treatment of experiment 14 was clearly better than that of experiment 13, the standard treatment. The same difference was noted for callusing, but in the opposite direction. It seems to be indicated that while certain preliminary water treatments were beneficial for rooting, callusing was retarded, even by comparatively moderate water treatments, and this is spite of the indications already referred to showing that low oxygen supply (quite obviously associated with high water supply) favors callusing.

The excessive preliminary water treatments tested (experiments 15 and 16) showed retardation with respect to both rooting and callusing, but this effect was much more pronounced in the case of callusing.

In these experiments, as is true in general, base callus tended to be somewhat more pronounced than top callus when both were vigorous. But experiments 15 and 16, with excessive preliminary water treatments and low values for callus throughout, showed top callus surpassing base callus for the maintained temperature about 29° C. It

seems that the extra and excessive water treatment of the bases in these two cases retarded callus formation at both top and base, but not so pronouncedly at top as at base, where the water was applied. Exactly in what way this water treatment acted to retard or inhibit callus formation can not be stated. There are some reasons for believing that the presumable excess of water supplied to the tissues did not operate merely to retard movement of oxygen. The prolonged soaking may have removed some materials from the basal tissues, or other things may have happened. Almost all the cuttings of experiment 3, which had their bases surrounded by water (wet cotton) throughout the entire experiment period, failed to produce any callus at either base or top. The base of each cutting was subjected to continuous soaking, more prolonged than in the case of experiments 15 and 16. On the whole, the retardation in callus production seemed to vary directly with the length of the soaking period, as has been noted.

SUMMARY OF RESULTS OF EXPERIMENTS 1 TO 16

The main numerical results of callusing, especially with regard to the cardinal temperatures, are summarized in Table 2 along with the corresponding data for rooting. The minimum temperature for callusing was several degrees higher than the minimum for rooting. In the case of rooting some activity was observed at the lowest temperature in every experiment started after the middle of January, but in the case of top and base callusing the cuttings used in the 16 experiments gave no evidence of activity at the lowest temperatures.

The minimum temperature for base-callus formation was not far from 16° C.; no callus was observed on cuttings when any temperature below 16.2° was used, and the amount of callus that occurred at any temperature below 20° had in no case an index value higher than 3.

In most of the experiments the optimum for base-callus formation was 29° C. The corresponding optimum for rooting lay between 26° and 28°; hence the optimum for base callusing was slightly higher than that for rooting.

Except in experiments 3, 8, 15, and 16, in each of which the cuttings had been given some excessive water treatment, the maximum temperature for base callusing was about 38° C. The maximum temperature was about the same for base callusing and rooting. Experiments 5 and 6 are exceptions to this, however, for in these cases the maximum temperature for rooting was lowered to about 34°, but no such effect was evident in the case of callusing. These exceptions are probably due to the low oxygen supply in these two experiments. The minimum oxygen supply was higher for rooting than for callusing.

In general, base callusing was more pronounced than top callusing, and this seems in some measure to have been responsible for the narrower temperature range indicated for top callusing. When nontemperature conditions are considered as not limiting, the apparent range for top callus is about 20° to 38° C., whereas that for base callus is about 16° to 38°. Whether the upper limit of these two apparent ranges (38°) is really the upper limit of the absolute range

for each process can not be definitely stated, but it seems likely that 38° is approximately the highest temperature at which any callus formation at either end of the cutting would occur, no matter what length of period was employed.

Apparently, no top callusing took place at any temperature below 20.3° C., and the most that occurred at any temperature below 23° is represented by an index value of 4 (experiment 4); thus the minimum temperature for formation of top callus was not far from 20° in these experiments. On the other hand, the minimum for the formation of base callus was about 16°.

The absence of callus on the cuttings of experiment 3 and on those at some of the maintained temperatures of experiments 15 and 16 does not imply that base callus can not ultimately develop when the cutting bases are water-logged, for considerable base callus was observed to have formed under water in some long-time experiments not here reported.

The studies of Knight (9) indicate that callusing was actually favored by water in his tests, for he observed more callusing in wet clay soils than in drier sandy soils. Knight's observations extended over several months rather than a few days, and in these experiments also the time factor seems to have played an important rôle. The observations of Knight seem to be in agreement with those of the present writer, if the suggestions be accepted that callusing is greatly retarded, but not inhibited by excessive amounts of water, and that the most important part played by sand in retarding callusing comes from its obviously very much higher oxygen supply.

Some evidence was secured indicating a seasonal relation of callusing similar to that shown for rooting (fig. 3) although this relation was not seriously studied for either callusing or rooting. For like treatments, experiment 13 (begun February 26) gave generally higher callus values than did experiment 4 (begun December 7). It appears that both rooting and callusing are more vigorous in the spring than in the fall, other conditions being similar and favorable, and of course the same is known to be true for the pushing of buds in such forms as apple.

No effect of buds upon the rooting or callusing of cuttings was observed. It appeared that rooting was confined entirely to the primordia visible on the cutting at the time it was taken from the tree, and that most of these primordia rooted, irrespective of the presence or absence of buds on the cutting. In almost all the tests callusing appeared to be closely associated with rooting, with certain environmental differences indicated, however, for optimal rooting and optimal callusing. A slightly higher temperature, a somewhat lower water supply, and a somewhat lower oxygen supply seemed to be required for optimal callus formation than for optimal root formation.

ADDITIONAL EXPERIMENTS ON ROOTING AND CALLUSING OF APPLE CUTTINGS

Several additional experiments with apple cuttings were performed, in some of which gas did not flow continuously through the experiment chambers. In these tests, chambers of different sizes or gas mixtures with different percentages of oxygen were used, the number of cuttings being the same in the different chambers of any series. In other cases flowing air was used, or the air of the chamber was renewed from time to time. In some experiments several different maintained temper-

atures were employed, and in others the temperature was the same for all chambers. Several different water treatments were used.

Three of these additional experiments (Nos. 17, 18, and 19) were performed with temperature series similar to those used in experiments 1 to 16. For these experiments, sealed chambers of about 1,000 c. c. capacity were used. The chambers were filled with a known gas mixture when the cuttings were inserted, and there was no gas flow. For experiment 17 the gas mixture was half oxygen and half nitrogen, for experiment 18 it was ordinary air, and for experiment 19 it was 90 per cent nitrogen and 10 per cent oxygen. All cuttings were soaked for one hour before they were placed in chambers.

The numerical data from these three experiments are presented in Table 3 and are graphically shown in the lower part of Figure 10. These results seem to emphasize further the suggestion obtained from the experiments with flowing gases: namely, that the oxygen supply requisite for optimum rooting or callusing is different for different temperatures. With the lowest oxygen supply (the 10 per cent mixture) the maximum temperature was about 23° C., but for the other two gas treatments (21 per cent and 50 per cent oxygen) the maximum was about 37°. For the lowest supply of oxygen, the optimum was about 17°, and for the two other gas treatments a broad optimal range of temperatures is evident, extending as far as 32° for the 50 per cent mixture.

TABLE 3.—Numerical values for pushing of roots and growth of callus of apple cuttings in sealed chambers, without flowing gas

Experiment No.	Conditions	Temperature	Callus				Temperature	Roots	Callus				Temperature	Roots	Callus			
			Roots	Top	Base	Lenticel			Top	Base	Lenticel	Temperature			Roots	Top	Base	Lenticel
17	50 per cent O ₂ -----	°C.	2	0	0	3	°C.	8	0	0	5	°C.	10	0	0	2	8	
18	Air-----	12.4	5	0	0	3	16.3	10	0	0	5	20.4	9	0	0	2	7	
19	10 per cent O ₂ -----	12.4	4	0	0	3	16.3	9	0	0	5	20.4	8	0	0	2	5	

Experiment No.	Conditions	Temperature	Roots	Callus				Temperature	Roots	Callus				Temperature	Roots	Callus				Temperature of highest chamber (no growth)					
				Top	Base	Lenticel				Top	Base	Lenticel				Top	Base	Lenticel							
17	50 per cent O ₂ -----	°C.	10	0	3	9	°C.	10	0	3	9	°C.	10	0	0	9			°C.						
18	Air-----	23.5	10	2	4	9	28.3	10	2	6	9	31.9	7	0	0	4			36.7						
19	10 per cent O ₂ -----	23.5	0	0	0	2	28.3	0	0	0	2	31.9	0	0	0	2			36.7						

Four additional experiments (Nos. 20, 21, 22, and 23) involved several different water and gas treatments, all carried out in the dark room at a temperature of about 19° C. All of these experiments began February 5, 1927, and for each one 23 cuttings were used. The several treatments and the rooting score values are shown in Table 4. It seems to be clearly indicated here, as in the results of experiments 3 and 8, that any considerable amount of wetting was detrimental to root pushing.

TABLE 4.—*Effect of water treatment on pushing of apple roots at about 19° C.*

[Started on February 5; each test contained 23 cuttings]

Experiment No.	Preliminary treatment	Gas treatment	Roots (scale of 10)
20	None.....	Flowing, saturated air.....	9
21	do.....	Moist, open air; bases occasionally wetted.....	8
22	Soaked 24 hours...	Flowing, saturated air.....	1
23	None.....	Nearly saturated air, changed daily; cuttings immersed 10 minutes daily.	.5

Two other experiments (Nos. 24 and 25) were also conducted in the dark room at a temperature of about 19° C., beginning March 4, 1927. Sealed chambers of different sizes were used, without any change of gas during the experiment period. In experiment 24 the chambers were originally filled with air, and in experiment 25, with oxygen. Twenty-five cuttings were used for each chamber, their bases having been soaked 24 hours before the test started. Each experiment gave comparisons between five different sizes of chambers. The volumes of the chambers and the score values for root pushing and for the production of top, base, and lenticel callus, as secured at the end of the 10-day period, are given in Table 5.

TABLE 5.—*Numerical values for pushing of roots and for growth of callus in apple cuttings in sealed chambers, with different gas mixtures, at a temperature of about 19° C.*

[Started on March 4; the bases of all cuttings were soaked 24 hours]

Experiment No.	Gas used	Volume of sealed chamber	Roots	Callus		
				Top	Base	Lenticel
		C. c.				
24-A	Air.....	5,400	10	0	3	8
24-B	do.....	3,000	10	0	3	8
24-C	do.....	2,700	10	0	2	6
24-D	do.....	1,800	7	0	1	4
24-E	do.....	1,000	1	0	0	2
25-A	Oxygen.....	5,400	10	0	0	7
25-B	do.....	3,000	9	0	0	6
25-C	do.....	2,700	9	0	0	5
25-D	do.....	1,800	10	0	0	3
25-E	do.....	1,000	3	0	0	2

In addition to offering indications as to the supply of oxygen necessary for optimum root pushing, these data show (1) that more base callus than top callus was formed and (2) that large supplies of oxygen had retarded callus formation at both ends of the cutting.

In experiment 26 (started October 18) the chambers used were large-mouthed bottles. The air in one bottle was swept out by oxygen, and a rubber bag containing oxygen under slightly more than atmospheric pressure was then connected and allowed to remain throughout the test; a second bottle was arranged in the same way, but oxygen was passed through it for a short period every day; the third bottle was filled with air at the start and was left with two small openings through which some gas exchange with the external air might occur. All cuttings rested with their bases upon damp cotton, and all bottles stood on a bench in the greenhouse without intense

sunlight. After three weeks all visible primordia had pushed except those in the sealed bottle with oxygen; here only a trace of activity was observed. It seemed quite evident that lack of oxygen rather than surplus of oxygen was responsible for this marked inhibition of rooting.

In two preliminary experiments the apple cuttings in some chambers were subjected to carbon dioxide (from a commercial tank) with nearly an atmosphere of pressure, while in other chambers the cuttings were subjected to nearly pure nitrogen. Both of these treatments showed injury as indicated by tissue disintegration in the bark and wood, but the cuttings treated with carbon dioxide were much more seriously injured than the others. In both cases injury was especially pronounced and rapid in the series of cuttings which bore leaves. Here, as in those without leaves, injury was evident sooner with carbon dioxide than with nitrogen.

The results of these additional experiments seemed to support the general conclusions reached in the main series, namely, that apple cuttings have a higher oxygen requirement for rooting than for callusing, and that any study of the oxygen supply required for rooting or callusing must include special consideration of the temperature. The experiments without flowing gas are to be considered as only superficial in their indications, because of the fact that the nature of the gas mixture undoubtedly changed during the experiment period and in an unknown manner. The oxygen content must have been decreased more or less rapidly through the respiration of the cuttings themselves and perhaps to a considerable degree by the oxidation occurring on the iron wire used as supports.

EXPERIMENTS WITH WILLOW CUTTINGS

The studies on willow cuttings were about equally divided between experiments with flowing gases and experiments with sealed chambers. The experiments with flowing gases will be considered first. Willow cuttings were present in the chambers of the first 12 experiments with apple cuttings.

The numerical results of these 12 experiments on willow are presented in Table 6. In addition to the numerical scores for rooting, derived as has been described for the experiments with apple cuttings, the results for willow include another value representing rooting activity, namely, the average number of roots per cutting evident at the end of the experiment. These additional values are given in the table in parentheses following the regular score values. Thus for experiment 5, for temperatures 20.6°, 23.6°, 28.6°, and 32.8° C., the root scores are, respectively, 1, 4, 5, and 5, and the new root values (in parentheses) are 0.3, 1.1, 1.7, and 1.0. In the third case, for instance, the score 5 indicates that half of the cuttings showed some root pushing, and the value 1.7 is the quotient obtained by dividing the total number of roots observed by the number of cuttings used.

Table 7 gives the numerical results of an experiment run at the same time as experiment 3. Here, however, only one temperature was used, and different rates of air supply were compared. These results will be considered only as regards callus formation, for rooting was similar in this case to that in experiment 3.

TABLE 6.—Numerical values for pushing of roots and for growth of callus of willow cuttings

Experiment No.	Date started	Conditions	Pushing of roots ^a			Callus ^b			Roots ^c			Callus ^b			Roots ^c			Callus ^b			Roots ^c			Temperature of highest chamber, no.									
			Temp. °C.	Top	Base	Temp. °C.	Top	Base	Temp. °C.	Top	Base	Temp. °C.	Top	Base	Temp. °C.	Top	Base	Temp. °C.	Top	Base	Temp. °C.	Top	Base										
1	1926 Nov. 8	Flowing O ₂ ; bases on damp cotton	7.7	0	0	0	0	14.7	0	0	0	220.7	4	(-)	(+)	827.7	9	(-)	(+)	834.1	5	(-)	(+)	339.8	0	0	0	46.5					
2	Nov. 23	Flowing air; bases on damp cotton	13.2	0	0	0	0	17.2	0	0	0	121.9	5	0	1	425.7	7	1	2	531.3	1	8	9	0	36.0	1	0	5	40.7				
3	Dec. 7	Flowing air; bases on wet cotton	14.1	0	0	0	0	217.8	0	0	0	422.1	10	0	0	625.5	10	0	0	830.3	10	1	0	835.3	10	0	0	6	40.2				
4	do.	Flowing air; bases on wire stilts	14.1	0	0	0	0	117.8	0	1	0	422.1	6	5	5	625.5	10	5	5	630.3	8	5	3	535.3	1	2	3	2	40.2				
5	Jan. 5	Flowing 5 per cent O ₂ ; fast; stilts	13.3	0	0	0	0	116.7	0	0	0	120.6	1	0	0	223.6	4	(1.1)	3	5	323.6	5	(1.7)	6	432.8	5	(1.0)	5	4	37.9			
6	do.	Flowing 5 per cent O ₂ ; slow; stilts	13.3	0	0	0	0	116.7	0	0	0	220.6	2	(0.7)	0	2	623.6	7	(2.5)	5	5	828.6	5	(2.1)	5	5	832.8	9	(3.0)	5	5	37.9	
7	Jan. 21	Flowing O ₂ ; stilts	14.4	0	0	0	0	117.9	0	0	0	221.7	0	(0.3)	1	2	324.8	3	(0.8)	2	3	329.7	3	(1.2)	1	0	333.8	1	(0.2)	0	1	38.9	
8	Feb. 3	Flowing air; stilts; injected 22 hours	14.2	0	0	0	0	547.7	8	(3.0)	0	621.4	10	(10.0)	0	5	724.5	10	(15.7)	3	5	829.6	10	(18.4)	2	5	832.7	10	(9.5)	0	3	39.0	
9	do.	Flowing air; stilts; bases soaked 22 hours	14.2	0	0	0	0	417.7	6	(1.5)	0	521.4	9	(5.2)	0	4	724.5	10	(12.1)	4	7	729.6	10	(12.3)	5	8	733.7	10	(13.7)	0	2	39.0	
10	Feb. 15	Flowing air; stilts; injected 15 hours	12.4	0	0	0	0	316.2	6	(1.7)	0	520.3	10	(14.7)	0	0	623.4	10	(14.7)	0	4	628.6	10	(21.2)	0	6	632.8	10	(18.0)	0	5	(-)	37.6
11	do.	Flowing air; dry 22 hours; injected 25 minutes	12.4	0	0	0	0	316.2	7	(2.3)	0	520.3	10	(12.8)	0	0	623.4	9	(17.3)	0	4	628.6	10	(19.7)	0	6	632.8	10	(20.0)	0	5	(-)	37.6
12	do.	Flowing air; dry 22 hours; injected 25 minutes	12.4	0	0	0	0	316.2	6	(2.6)	0	520.3	10	(16.3)	0	2	623.4	9	(9.6)	0	4	628.6	10	(19.5)	0	6	632.8	6	(10.3)	0	5	(-)	37.6
		Average of 11 and 12	12.4	0	0	0	0	316.2	6.5	(2.5)	0	520.3	10	(14.6)	0	1	623.4	9	(13.5)	0	4	628.6	10	(19.6)	0	6	632.8	8	(15.2)	0	5	(-)	37.6

^a The figures in this column represent the proportion of cuttings that showed some pushing of roots at the end of 10 days, calculated on the basis of 10. The figures in parentheses show the average number of roots per cutting evident at the end of the 10-day period.

^b The figures in these columns represent the total amount of callus shown by all the cuttings in each lot (not the number of cuttings that showed some callus), based on an arbitrary scale of 10.

^c No observation.

TABLE 7.—Numerical values for pushing of roots and growth of callus of willow cuttings with a maintained temperature of 27° C. and with different rates of flowing gas

[Started on December 7, 1926; all cuttings with wet cotton around bases]

Experiment	Speed of air flow	Roots	Callus		
			Top	Base	Lenticel
A	300 c. c. per hour.....	10	0	0	7
B	50 c. c. per hour.....	10	2	0	7
C	20 c. c. per hour.....	10	6	0	7
D	10 c. c. per hour.....	9	6	1	7

Table 8 shows the data from all experimentation with the seven different maintained temperatures when flowing gases were not used; each chamber was filled with the gas indicated and then sealed. Experiment 7-A is so numbered because it was run at the same time as experiment 7. Experiments 13 to 16 were all started February 26, 1927.

TABLE 8.—Numerical values for pushing of roots and for growth of callus of willow cuttings with maintained temperatures, without flowing gases

Experiment No.	Gas at start	Date started	Treatment	Callus				Callus				Callus			
				Temperature	Roots	Top	Base	Temperature	Roots	Top	Base	Temperature	Roots	Top	Base
7-A	Oxygen	1927 Jan. 21	Stilts	°C.				°C.				°C.			
13	Air	Feb. 26	do	14.4	0	0	0	17.9	1	0	0	21.7	4	0	0
14	do	do	do	12.2	0	5	5	16.2	1	8	8	20.3	2	10	10
15	do	do	do	12.2	0	0	0	16.2	1	6	0	20.3	1	9	0
16	do	do	do	12.2	0	0	0	16.2	1	0	0	20.3	1	3	3
	do	do	do	12.2	0	0	0	16.2	1	2	2	20.3	4	3	3

Experiment No.	Gas at start	Date started	Treatment	Temperature	Callus			Temperature	Callus			Temperature	Callus			Temperature of highest chamber; no growth
					Roots	Top	Base		Roots	Top	Base		Roots	Top	Base	
7-A	Oxygen	1927 Jan. 21	Stilts	°C.				°C.				°C.				°C.
13	Air	Feb. 26	do	24.8	3	1	3	29.7	5	2	2	33.8	0	0	0	38.9
14	do	do	do	23.7	3	10	10	28.7	2	8	9	32.8	1	5	5	38.0
15	do	do	do	23.7	2	10	2	28.7	2	8	0	32.8	1	5	0	38.0
16	do	do	do	23.7	3	3	4	28.7	1	3	4	32.8	0	0	0	38.0
	do	do	do	23.7	4	3	4	28.7	1	6	4	32.8	1	3	4	38.0

Table 9 presents the numerical results of all experimentation with willow in which the single-chamber constant-temperature apparatus was used except those given in Table 7. In none of the experiments referred to in Table 9 was flowing gas used; sealed chambers were employed in all cases, though in 4 of the 39 tests gas was passed through the chamber twice daily. In each of these tests the experiment period was seven days.

TABLE 9.—Numerical values for pushing of roots, for average number of roots per cutting, and for growth of callus of willow cuttings, with maintained temperatures and with different gas supplies

[All cuttings were on wire stilts, 50 cuttings in each lot]

Experiment No.	Date started	Temperature	Preliminary treatment	Size of bottle	Gas treatment	Roots		Callus		
						Cuttings rooted	Average roots per cutting	Top	Base	Lenticel
	1927	° C.		C. c.						
17-A	Jan. 8	27	None	4,200	Air, closed	6	2.5	2	3	3
17-B	do	27	do	4,200	Air, changed twice daily	2	.7	5	6	4
17-C	do	27	do	4,200	O ₂ , closed	5	1.2	1	2	3
17-D	do	27	do	4,200	O ₂ , changed twice daily	3	.8	0	0	2
18-A	Jan. 15	27	do	4,200	5 per cent O ₂ , closed	0	.3	0	0	4
18-B	do	27	do	4,200	5 per cent O ₂ , changed twice daily	2	.7	3	5	5
18-C	do	27	do	4,200	N ₂ , closed	0	.1	0	0	1
18-D	do	27	do	4,200	N ₂ , changed twice daily	0	.2	0	0	2
19-A	Jan. 22	27	do	4,200	Air, closed	8	5.5	5	6	7
19-B	do	27	do	4,200	29 per cent O ₂ , closed	7	3.7	5	6	7
19-C	do	27	do	4,200	38 per cent O ₂ , closed	8	3.7	5	6	7
19-D	do	27	do	4,200	12 per cent O ₂ , closed	1	.5	3	4	7
20-A	Jan. 29	26.5	do	4,200	Air, closed	8	3.7	5	6	7
20-B	do	26.5	do	4,200	21 per cent O ₂ , closed	9	3.3	5	6	7
20-C	do	26.5	do	4,200	17.5 per cent O ₂ , closed	3	1.0	4	5	7
20-D	do	26.5	do	4,200	14 per cent O ₂ , closed	1	.5	3	4	7
21-A	Feb. 5	29	do	4,200	Air, closed	7	4.9	5	6	7
21-B	do	29	do	4,200	50 per cent O ₂ , closed	7	4.4	5	6	7
21-C	do	29	do	4,200	75 per cent O ₂ , closed	8	6.9	4	5	7
22-A	Feb. 12	29	do	5,400	Air, closed	6	3.7	7	8	7
22-B	do	29	do	4,200	do	5	3.0	7	8	7
22-C	do	29	do	2,700	do	2	.5	6	7	7
22-D	do	29	do	2,200	do	3	.9	7	8	7
23-A	Feb. 19	29	Injected	5,400	do	10	11.6	0	3	7
23-B	do	29	do	4,200	do	10	7.4	0	2	7
23-C	do	29	do	2,700	do	1	.7	0	1	7
23-D	do	29	do	2,200	do	0	.5	0	0	7
24-A	Feb. 27	29	Dry two days.	4,200	do	6	3.0	5	6	7
24-B	do	29	do	4,200	50 per cent O ₂ , closed	8	6.6	5	7	7
24-C	do	29	do	4,200	75 per cent O ₂ , closed	8	7.8	5	7	7
24-D	do	29	do	4,200	O ₂ , closed	9	7.1	5	7	7
25-A	Mar. 6	32	None	4,200	Air, closed	5	2.9	9	10	7
25-B	do	32	do	4,200	50 per cent O ₂ , closed	8	6.7	9	10	7
25-C	do	32	do	4,200	75 per cent O ₂ , closed	8	6.0	9	10	7
25-D	do	32	do	4,200	O ₂ , closed	9	7.0	9	10	7
26-A	Mar. 13	34.5	do	4,200	Air, closed	1	.6	7	8	7
26-B	do	34.5	do	4,200	50 per cent O ₂ , closed	4	1.9	7	8	7
26-C	do	34.5	do	4,200	75 per cent O ₂ , closed	4	2.7	5	6	7
26-D	do	34.5	do	4,200	O ₂ , closed	4	2.5	5	6	7

As in the case of the apple data, the results with willow, especially those with maintained temperatures and flowing gases, will be discussed mainly with reference to the corresponding graphs, Figures 11 to 17. In those graphs (figs. 15, 16, and 17) in which broken lines are used to represent average number of roots per cutting, the number representing the greatest average amount of root growth observed in any of these 12 experiments (21.2) has been taken as equal to 10 on the scale.

ROOTING OF WILLOW CUTTINGS

In experiment 1 (fig. 11, 1R), in which flowing oxygen was used, the total temperature range employed was much wider than the range of rooting, so that of the experiments at the seven temperatures only three showed any growth. The graph shows an optimum at 27.7° C..

but it may have been several degrees higher or lower. The maximum appears to have been about 39° or below, while the minimum temperature is indicated as above 15° , probably below 18° .

In experiment 2 (fig. 11, 2R) the conditions of the test were similar to those of experiment 1 in that the bases of the cuttings rested on damp cotton, but in this case flowing air was used. The graph for experiment 2 shows an optimum at about 26° C., but from the data the optimum might just as well have been 28° . The maximum was about 40° or below. The minimum was slightly higher than that shown by experiment 1, being above 17° or possibly above 19° .

In experiment 3 (fig. 12, 3R) flowing air was used, and the bases of the cuttings, instead of resting on moist cotton, were surrounded to a height of about 5 cm. by wet cotton. This additional water seems to have produced a beneficial effect upon rooting, so that although maximum and minimum temperatures are indicated as before, no definite optimum temperature is apparent; the entire graph between the maximum and the minimum is thus raised to the top, for every cutting in each of the four chambers between 22.1° and 35.3° C.

showed pushing of roots. However, notes on this experiment show that among the four temperatures more roots per cutting as well as longer roots were present at 30.3° than at 25.5° . With respect to these criteria, 22° seemed to be about equal to 35° , but with both the latter temperatures less rooting occurred than with 25.5° . Therefore, it seems that the additional water treatment of experiment 3 greatly increased rooting within the total temperature range, but did not appreciably alter the previously observed maximum, optimum, or minimum, here seen to be about 39° , 29° , and 18° , respectively.

Experiment 4 (fig. 12, 4R) was a duplicate of experiment 3, except that in experiment 4 the cuttings were supported on wire stilts so that no water came in contact with the cuttings. Again there is a temperature curve similar to the other curves discussed, with maximum and minimum not far from 40° and 18° C., respectively, and with an optimum indicated at 25.5° , but which may well have been

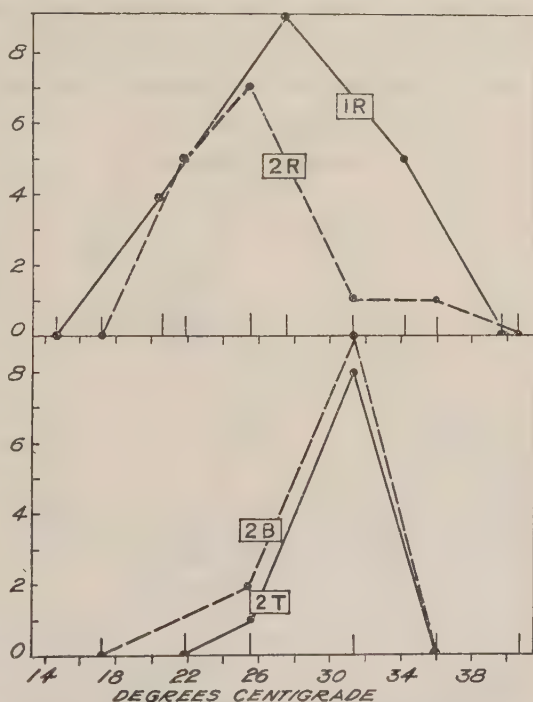


FIGURE 11.—Rooting (1R, 2R), top-callus formation (2T), and base-callus formation (2B) of willow cuttings in closed chambers with flowing oxygen (experiment 1) or with flowing air (experiment 2)

about 29°. While all cuttings did not root at 30.3°, this temperature was here, as in experiment 3, a slightly better one for the subsequent growth of the roots that did push than was 25.5°. Although the curve is for some unknown reason higher in experiment 4 than in experiment 2, still rooting in experiment 4 was much less than that observed in experiment 3, where the cuttings quite certainly were furnished with some water from the cotton. Apparently the beneficial effects of the water treatment were greatest at the supra-optimal temperatures rather than at the sub-optimal temperatures, as was observed in the corresponding experiment for apple rooting.

In experiments 5 and 6 (fig. 13) all cuttings were on wire stilts, and a flowing gas mixture containing 5 per cent oxygen was used. A

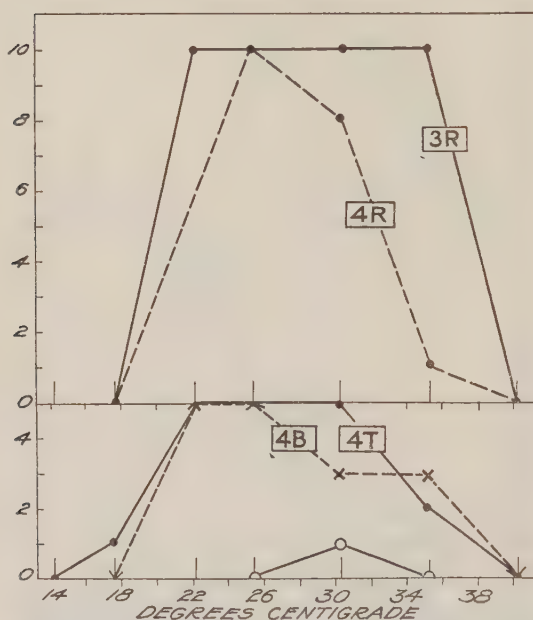


FIGURE 12.—Rooting (3R, 4R), top-callus formation (4T), and base-callus formation (4B) of willow cuttings in closed chambers with flowing air: Experiment 3, with the bases of the cuttings on wet cotton; experiment 4, with the bases on wire stilts. With the wetter conditions around the cuttings of experiment 3, only a trace of callus appeared

rate of flow of about 300 c. c. per hour was employed in experiment 6, while a rate of about 50 c. c. per hour was used in experiment 5. In marked disagreement with the corresponding curve for apple rooting (fig. 3), the very low oxygen supply in experiment 6 at 33° C. appeared ample for good rooting in willow. In fact, except for the depression at 28°, the graph for experiment 6 is not unlike the other graphs for willow cuttings that were subjected to flowing gases without having been given some water treatment.

Another interesting point shown by Figure 13 is the fact that the graph for experiment 5 lies wholly below that

for experiment 6. Supposing that the lots of cuttings for the different tests were similar, the most obvious differences between the conditions for experiments 5 and 6 are differences in amounts of water vapor and oxygen in the corresponding chambers, due to different rates of gas flow. It seems unlikely that there was any difference in the humidity of the gas about the cuttings in the two experiments. There was surely a much larger supply of oxygen in experiment 5 than in experiment 6, and it is possible that excess of oxygen may have slightly retarded rooting in the former experiments. There are, however, several possible objections to this suggestion; for example, the results of experiment 1 (with flowing oxygen) and of experiment 4 (with flowing air) appear to be in disagreement with it. It is possible that the presence of the wet cotton in experiment 1 may, in some

way, have counteracted unfavorable effects of high oxygen concentrations. This possibly injurious effect of large supplies of oxygen is also suggested in experiment 7 and in some of the experiments listed in Tables 8 and 9, in which continuously flowing gases were not used. On the basis of the supposition that large supplies of oxygen produce an injurious effect upon rooting of willow, it is hard to reconcile the results of experiment 4, where large quantities of air were passed through the chambers, where no liquid water was supplied the cuttings, and where, nevertheless, high percentages of rooting were secured.

In contrast to the corresponding results with apple, the willow cuttings in experiment 6 gave no evidence of oxygen deficiency at supra-optimal temperatures; the small amount of oxygen here supplied seemed to have been ample for good root growth, even at 32.8° C. Except with regard to the somewhat questionable optima, the graphs for experiments 5 and 6 agree with the other graphs for willow, showing maxima near 38° and minima at about 17°.

The results of experiment 7, as just mentioned, suggest that a large supply of oxygen may be detrimental to the rooting of willow cuttings. Although the usual maximum of about 38° C. is here indicated, experiment 7 (fig. 14, 7R) shows the only graph obtained in all these 12 experiments in which the minimum is above 18°; here the minimum is about 22°. The optimum (about 25°) is likewise unusual. All score values in this experiment are low, which seems to support the suggestion that an excess of oxygen may retard rooting in willow.

The striking differences brought out in Figure 14, between the results of experiment 1 (flowing oxygen, started November 8) and those of experiment 7 (flowing oxygen, started January 21) hardly seem to have been the result of seasonal differences in the capacity of the cuttings to produce roots. They seem rather to have been related either to unlike variability in the two lots of cuttings or to differences in water supply; the cuttings in experiment 1 may have

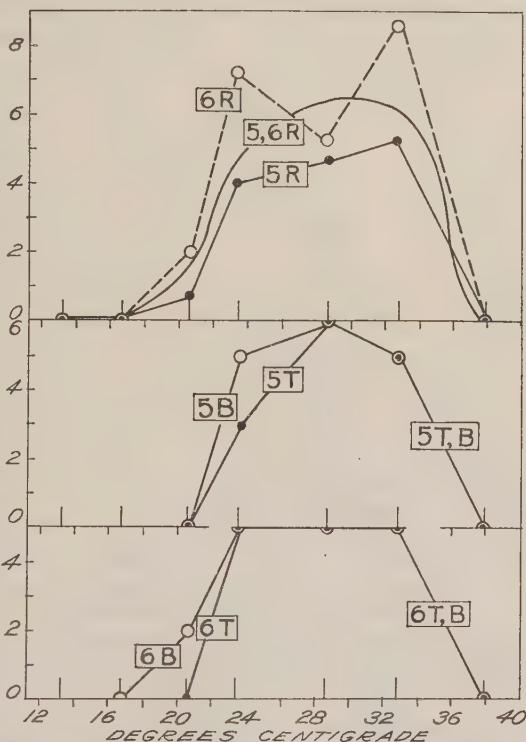


FIGURE 13.—Rooting (5R, 6R), top-callus formation (5T, 6T), and base-callus formation (5B, 6B) of willow cuttings in closed chambers with flowing 5 per cent oxygen in nitrogen

been supplied with sufficient water to counteract any injurious effect caused by surplus of oxygen.

In experiments 8 and 9 (fig. 15) the maxima and minima were similar to those shown by most of the other willow experiments (about 39° and 15° C., respectively). But in these two experiments, as in experiment 3, where also the cuttings had been treated with water, the optima are not clear, for all score values for temperatures below the maximum are 10 or only slightly below. Nevertheless, by considering the average number of roots per cutting the optima are seen to lie in the vicinity of 28° . (The broken lines—marked 8r and 9r in

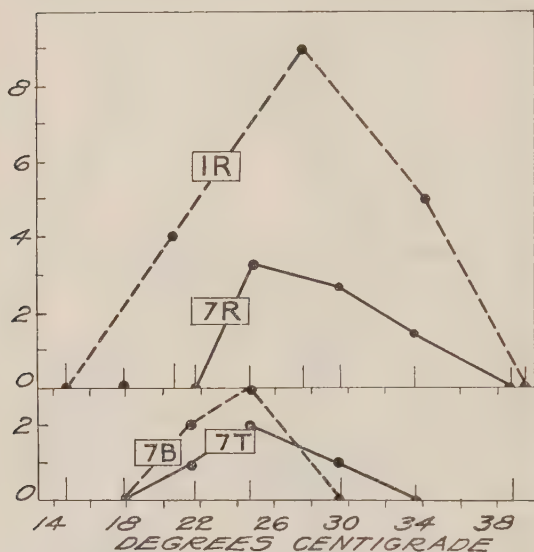


FIGURE 14.—Rooting (1R, 7R), top-callus formation (7T), and base-callus formation (7B) of willow cuttings in closed chambers with flowing oxygen. Experiment 1, bases on damp cotton; experiment 7, bases on wire stilts

willow cuttings were benefited directly in proportion to the intensity and length of the water treatment used. The only reason for supposing that prolonged water treatments at high temperatures may have been injurious (as, for example, because of lack of oxygen) arises from the fact that a slightly greater number of roots per cutting were shown by experiment 9, as compared with the number in experiment 8, at the highest temperature at which rooting occurred (33.7°). Although this difference in number of roots per cutting ($13.7-9.5$) is not large, it is in the direction that might be expected from the results with apple; for it seems reasonable to suppose that rooting of willow may be retarded by lack of oxygen, even though the critical limit lies far below that for rooting of apple.

The cuttings in experiments 10, 11, and 12 showed more rooting than those in the experiments with willow. Of these cuttings, those in experiment 10 (fig. 16) were given the longest water treatment and showed the greatest root growth. In experiments 11 and 12 (fig. 17) the cuttings were kept dry for one day and then given short, intensive

fig. 15, 10r in fig. 16, and 11, 12r in fig. 17—indicate the total average number of roots per cutting, the greatest number obtained in any of these experiments—21.2 per cutting in experiment 10—being taken as 10 on the scale of ordinates used.) In contrast to results obtained with apple cuttings, willow cuttings completely soaked for 22 hours after a 15-minute injection gave decidedly higher rooting values than cuttings that had the bases only soaked for 22 hours; the latter treatment, however, gave much higher values than no water treatment at all. In fact, these

water treatment before being placed in the chambers; thus, whatever the total amount of water supplied to the cuttings by the two different treatments, at the end of the test the cuttings of experiment 10 had really been under rooting conditions for 10 days, as opposed to only 9 days for the others. Those used in experiments 11 and 12 received essentially identical treatments throughout, being run as duplicates in different chambers at the same temperatures.

With respect to maximum and optimum temperatures (about 37° and 29° C., respectively), the results of these three experiments (Nos. 10, 11, and 12) agree closely with those of most of the other willow tests. But these three experiments show lower minimum temperatures than any of the others with willow. A high-score value for rooting was obtained at 16.2° , and the minimum temperature for discernible root growth in 10 days lies not far from 13° .

Next to these low minima obtained in experiments 10, 11, and 12, started February 15, the lowest temperatures at which any rooting occurred were in experiments 8 and 9, started February 3; these experiments showed high-rooting values for 17.7° C. It is interesting to compare these results at a temperature of 17.7° in the two experiments last mentioned with the results at a similar temperature in experiment 3 (started December 7). The high-score values shown in both cases for other temperatures indicate that the water treatments used were satisfactory for good growth; but at a temperature of 17.8° in December no rooting whatever was evident, while at a temperature of 17.7° in February a large proportion of the cuttings rooted. As with the apple cuttings, it seems that some seasonal factor was involved which is evident in the graphs only in the vicinity of the minimum.

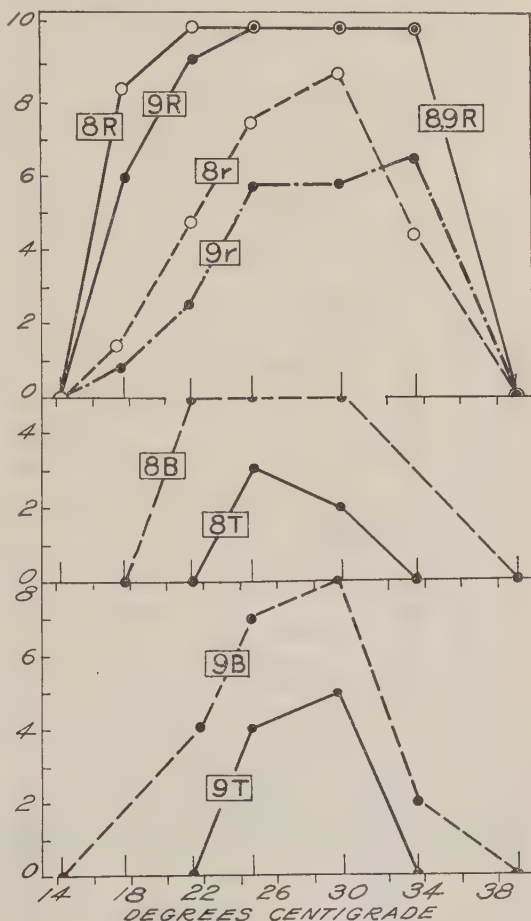


FIGURE 15.—Rooting (8R, 9R, 8r, 9r), top-callus formation (8T, 9T), and base-callus formation (8B, 9B) of willow cuttings in closed chamber with flowing air. Experiment 8, cuttings injected with water and completely immersed for 22 hours; experiment 9, bases of cuttings soaked for 22 hours

TABLE 10.—Summary of results of experiments 1 to 12 in rooting and callusing of willow cuttings

Ex- peri- ment	Date experi- ment started	Flowing gas	Water conditions	Data for rooting			Data for top callusing			Data for base callusing			Data for lenticel callusing		
				Approximate car- dinal tempera- tures			Approximate car- dinal tempera- tures			Approximate car- dinal tempera- tures			Approximate car- dinal tempera- tures		
				Minimum	Optimum	Maximum	Minimum	Optimum	Maximum	Minimum	Optimum	Maximum	Minimum	Optimum	Maximum
				° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.
1	1926 Nov. 8	Water saturated O ₂	Bases on damp cotton	17	27	40	9	(a)	(a)	(a)	(a)	(a)	10(?)	21-28	40
7	1927 Jan. 21	do	Cuttings not in contact with water.	22	25	38	3	18	25	29	3	10(?)	22-30	38	3
5	Jan. 5	Water-saturated mix- ture, 5 per cent O ₂	do	17	33	38	5	21	29	38	6	10(?)	29-33	38	4
6	do	do	do	17	33	38	9	21	23-33	38	5	10(?)	24-33	38	8
4	1926 Dec. 7	Water-saturated air	do	18	25	40	10	13	22-30	40	5	10(?)	22-25	40	6
2	Nov. 23	do	Bases on damp cotton	17	25	40	7	22	31	36	8	17	15	26	5
10	1927 Feb. 15	do	Injected, completely soaked 15 hours.	13	20-33	38	10	(b)	(b)	(b)	6	20	(a)	(a)	7
9	Feb. 3	do	Bases soaked 22 hours.	14	25-34	39	10	21	29	34	5	14	29	39	8
11, 12	Feb. 15	do	Dry 22 hours, injected 25 minutes.	13	20-30	38	10	(b)	(b)	(b)	6	16	29	38	6
3	1926 Dec. 7	do	Bases surrounded by wet cotton.	18	22-35	40	10	26	30	35	1	(a)	10(?)	25-30	40
8	1927 Feb. 3	do	Injected, completely soaked 22 hours.	14	21-34	39	10	21	25	3	3	18	21-29	39	8

* No observation.

† No callus.

SUMMARY OF RESULTS OF EXPERIMENTS 1 TO 12

The main numerical results of the experiments with willow cuttings in which flowing gases and maintained temperatures were used have been brought together in Table 10, as was done for the corresponding results with apple cuttings in Table 2.

The minimum temperature for rooting of willow apparently changed from about 18°C . early in the season to about 13° later in the season. On the other hand, the maximum temperature seems to have been at all times, whatever treatment was employed, about 38° , that is, about the same as that indicated for rooting and callusing of the apple. Except for the suggestion offered in experiments 8 and 9, apparently in none of these tests with willow did the supply of oxygen fall below the critical limit, even at the higher temperatures. Hence, from these particular experiments little is seen to indicate that the supply of oxygen necessary for rooting was in any way dependent upon the temperature. However, from the results obtained without flowing gases, which will be considered later, it appears that the failure of experiments 1 to 12 to show any definite effect of oxygen deficiency may be explained by supposing that the oxygen requirement for willow is very low and was satisfactorily met in all cases where flowing gases were used.

On the basis of the rooting scores the optimum temperature is not indicated so clearly as are the maximum and minimum, for at all temperatures between 20° and 33°C . rooting was very good; judged by the average number of roots per cutting, however, the optimum temperature was about 29° .

It should be emphasized that the minimum temperature for visible root growth in 10 days was several degrees higher for willow than for apple. Although much activity was observed in apple cuttings subjected to the lowest temperatures, in every series of willow cuttings the lowest temperature employed was too low for rooting.

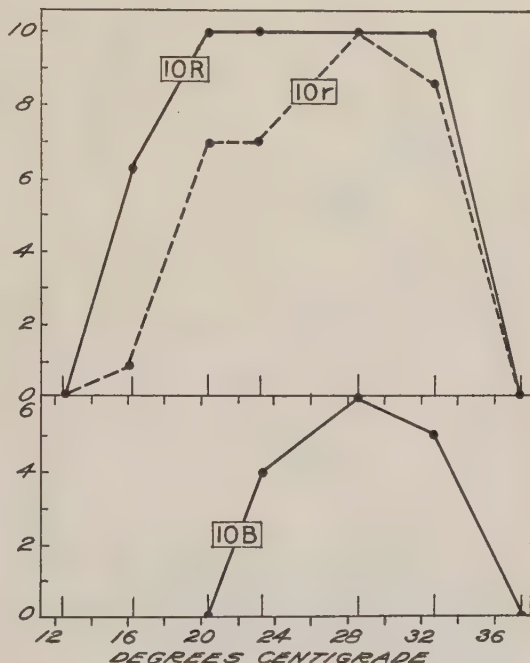


FIGURE 16.—Rooting (10R, 10r) and base-callus formation (10B) of willow cuttings in closed chambers with flowing air and with the cuttings injected with water and soaked for 15 hours at the start of the test. No top callus formed with this intensive water treatment

On the other hand, apple and willow were approximately alike in respect to their optimum and maximum temperatures. Although in these 12 experiments with flowing gases there was no indication of oxygen deficiency, the results of experiment 7 (the only experiment with flowing oxygen in which water was not available to the cuttings) suggested that the use of oxygen with a pressure of about 1 atmosphere (an oxygen excess) may have been instrumental in raising the

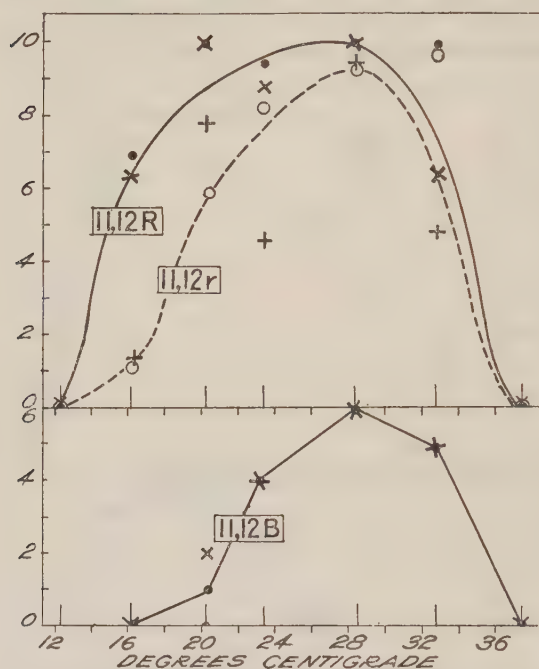


FIGURE 17.—Rooting (11, 12R; 11, 12r) and base-callus formation (11, 12B) of willow cuttings in closed chambers with flowing air, the cuttings having been injected with water after 22 hours of drying. No top callus formed with this treatment.

asmuch as all of the willow cuttings bore buds, these experiments differed from those with apple cuttings in that they did not furnish any evidence against the supposition just mentioned.

CALLUSING OF WILLOW CUTTINGS

The numerical data for callusing in willow cuttings in the experiments with flowing gases are shown by graphs based on Table 6.

Except for temperature influence, no important differences in callus formation are evident in the series of gas and water treatments employed in these 12 experiments. A graph for base-callus formation with a minimum of about 18° C., an optimum of about 29°, and a maximum of about 38° answers well for all these tests except experiments 3 and 7.

In experiment 3 the only callus observed on either end was a trace of top callus produced at a temperature of 30.3° C. In itself, this almost total absence of callus on cuttings that showed a very large amount of rooting suggests again that roots and callus may reciprocate, the

minimum temperature as well as in greatly decreasing the index values for all temperatures at which rooting occurred.

In regard to water supply, the results indicate that the more intense or prolonged the water treatment the greater was the rooting response. In this respect willow cuttings were very different from apple; some water treatments were highly beneficial for the rooting of apple cuttings, but more prolonged treatments were injurious.

In the experiments with willow no evidence was secured in support of the supposition that the presence of buds exerts any direct influence, as through hormones, upon root pushing. In-

activity being variously divided between rooting and callusing. However, a closer examination of the results with willow cuttings seems to indicate here, as with apple, that such reciprocation was not involved, but rather that there are actual differences between the requirements for rooting and for callusing. The bases of the cuttings in experiment 3 (being surrounded by wet cotton) were evidently too wet for callus formation, although such conditions were very good for root formation. The lack of top-callus formation in experiment 3 may, perhaps, have been due to excessive water treatment or to this water treatment in conjunction with the large oxygen supply. In the case of the special modification of experiment 3 in which different rates of air flow were employed at a single temperature (see Table 8), top callus was evident only with the lower rates of flow.

The score values for base callusing shown by experiment 7 are lower than the corresponding ones for any of the other experiments with flowing gases except experiment 3 and its modification. The extremely low maximum and optimum temperatures indicated by experiment 7, about 29° and 25° C., respectively, may have special significance, or they may show merely that all values were too low to give satisfactory temperature indications. These extremely low values for base callusing can not be explained either by a reciprocation of callusing with rooting or by excessive treatment with water. It seems that a surplus of oxygen was in some way responsible for them.

It may or it may not be significant that the only values higher than 6 for base callus were obtained in experiments 2 and 9 with cuttings that had been treated with water. While it may be that some treatment was beneficial to callusing of willow, it seems that even in willow cuttings any water treatment tended to retard callusing. This was especially evident in experiment 3 and in experiments 10, 11, and 12. In the last-mentioned experiments, although the index values for base callusing are about like those for the other experiments, top callusing was entirely inhibited by the injection treatment. In experiment 8, the other experiment in which the cuttings were injected under reduced pressure, only slight top callusing was evident; and in experiment 9, in which the cuttings were not injected but were soaked 22 hours, values for top-callus formation were more different from those for base callus than were the values shown by cuttings neither soaked nor injected. Thus in general, where the cuttings were given water treatment, the temperature range for formation of top callus lay well within the range for base callus, and in these cases the curves of minimum and maximum temperatures are not far from 21° and 34° C., respectively; also, the optima are uncertain, and all index values are low.

Except for the high value shown near 31° C. in experiment 2 and another near 29° in experiment 9, the conditions of these 12 experiments were not good for either base-callus or top-callus formation, whatever gas or water treatment was employed.

LENTICEL CALLUS

Other workers have made many studies of the external conditions influencing tissue hypertrophy around lenticels (lenticel callus). These studies have clearly demonstrated the close relationship existing between oxygen deficiency and water surplus in bringing about

lenticel hypertrophy when the temperatures are adequate, but apparently no special study has been carried out with reference to the part played by these different factors.

Although the system of score values for lenticel-callus used in the present studies is not wholly satisfactory, some indications with regard to lenticel-callus formation may be added here.

Regarding the temperature curve for lenticel-callus formation, the data presented in Tables 6 and 10 indicate maxima about 39° and minima about 10° C. The optima are not so definite, for in general little difference was shown for any one series within the temperature range between 22° and 30°.

Although the gas and the water treatments employed in these experiments seem to have had little influence upon the cardinal temperatures, the index values obtained were markedly influenced by such treatments. Experiment 1 shows that at proper temperatures lenticel callus may form with a high partial pressure of oxygen surrounding the cuttings. Considering the low values of experiment 7 (flowing oxygen, bases of cuttings on stilts) in contrast with the high values of experiment 1 (flowing oxygen, damp cotton around bases of cuttings), the external supply of oxygen seems to have been of much less importance in lenticel-callus formation than was the water supply. This suggestion is supported by the difference in results shown between experiments 3 and 4 and between experiments 8 and 9. When experiments carried out at the same time are considered, lenticel callusing was increased in proportion to the quantity of water supplied to the cuttings.

However, considering the differences between experiments 5 and 6 (both without water treatment) lenticel-callus formation seems to have been favored by a low oxygen supply; in experiment 6, in which the cuttings received only 50 cubic centimeters per hour of a mixture of 5 per cent oxygen, index values were markedly higher than those of experiment 5, in which the same gas mixture was passed through the chambers approximately six times as fast.

Thus these willow cuttings subjected at adequate temperatures to negligible rates of evaporation showed formation of lenticel callus with any gas mixture tested, whether supplied with water or not; but such hypertrophy was accelerated either when the partial pressure of oxygen was low or when water was supplied to the cutting.

ADDITIONAL EXPERIMENTS ON ROOTING AND CALLUSING OF WILLOW CUTTINGS

Some additional experiments on rooting and callusing of willow cuttings were made, the numerical results of which are shown in Tables 8 and 9. Experiment 7-A (Table 8) was a duplicate of experiment 7 (Table 6) except that instead of having continuously flowing oxygen the chambers were swept out with oxygen at the start of the experiment, and this was repeated 12 hours later, after which the chambers remained sealed. Unlike experiment 7, this experiment shows about the usual minimum rooting temperature (15° C.) and the usual optimum (about 29°). But the maximum temperature (33.8°) is lower than that in any of the willow experiments with flowing gases. At this temperature actual injury was sustained because of oxygen deficiency. Although more oxygen was introduced into each chamber in experiment 7-A than was supplied in experiment 6 (slowly flowing 5 per cent oxygen), rusting of the wire stilts

took place rapidly, and the available supply probably fell below the critical limit for rooting. It is uncertain whether in experiment 7-A the generally low rooting values were all due to deficiency in oxygen, or whether under some temperature conditions an optimum supply was available. The occurrence of the generally low callus values here shown suggests that injury may possibly have occurred at the lower temperatures because of too much oxygen.

The four experiments, Nos. 13 to 16, were all run at the same time (February 26 to March 8); each chamber contained air when sealed, and (except in experiment 13) some preliminary water treatment had been given to the cuttings.

In experiment 13 the low values for rooting and the very high values for callusing are in accord with previous suggestions that callus formation is favored by a very low oxygen supply and that, although the required oxygen supply for rooting of willow is low, it is not nearly so low as is that for callusing in the same plant. In this experiment, at every temperature employed, except perhaps near the minimum, the supply of oxygen fell during the experiment period below that required for good root formation. At 28.7° and 32.8° C., temperatures which other experiments have indicated as being near the optimum for callusing, the oxygen supply fell even below the low amount required for optimum callus formation. Consequently, the temperatures here indicated for optimum callusing (20.3° to 23.7°) are much below the optimum (about 29°) indicated by experiments 1 to 12.

The results of experiment 13 show that, for the suboptimal temperatures employed, conditions were very good for callusing of willow; in fact, better than in any other of the writer's experiments. The fact that at a temperature of 12.2° C. moderate callus was here observed at both top and base indicates that the temperature minima (about 18° to 21°) shown by the experiments with flowing gases were too high. Apparently in every case in experiments 1 to 12 willow callusing was in general greatly reduced because of excess of oxygen.

Experiment 14 (in which the bases of the cuttings were soaked 24 hours) showed rooting values as low as or lower than those for experiment 13 at the corresponding temperatures, indicating for willow (as has already been observed for apple) that, with deficiency of oxygen, injury was even more pronounced with wet cuttings than with dry ones. In this case callusing at the bases of the cuttings (the region soaked for 24 hours) was almost negligible, while the values for top-callus formation are very high—almost as high as those for experiment 13. That the almost total absence of base callus was not entirely due to the 24-hour soaking seems indicated by the fairly high callusing values shown in experiment 9 (Table 6), where, with flowing air, cuttings whose bases had been soaked for 22 hours showed more callus formation at the base than at the top.

In experiment 15 (in which water was injected into the cuttings for 25 minutes) and experiment 16 (in which the cuttings were soaked one hour) the values for callusing are again low, and the values for rooting are even lower. The available oxygen supply here also probably fell much below the critical limit for optimum rooting and even somewhat below that required for optimum callusing. Injecting the cuttings seems to have decreased rooting and callusing slightly more than immersing them for one hour; in experiment 15 the maxi-

imum temperature for both rooting and callusing was below 33° C. It is probable that the lower callusing activity shown by these last three experiments was not entirely due to oxygen deficiency, for indications that treatment of the cuttings with water tends to retard callusing, irrespective of the effect of such treatment on oxygen supply, have already been referred to.

In Table 9 are shown the results of two experiments (Nos. 17 and 19) with gas not under continuous flow, which again indicate that a large supply of oxygen may retard rooting. In experiment 17 air and oxygen were used, with chambers A and C remaining closed throughout the 7-day period and with the gas bubbled through chambers B and D every 12 hours. The cuttings in chamber A (air, closed) showed the highest rooting values of the four, those in chambers B (air, changed) and D (oxygen, changed) showing much lower rooting values. This experiment indicates that a large supply of oxygen was injurious, irrespective of the partial pressure employed. Experiment 19, A to D, also showed some indication that oxygen in excess may act injuriously upon the rooting of willow. Although the proportion of cuttings that rooted was not appreciably lower in chambers B and C than in chamber A, the lower average number of roots per cutting may have been due to higher oxygen content.

Table 9 also refers to many experiments with willow in which low rooting values seem clearly to have been due to deficiency of oxygen. In experiment 18, A to D, nitrogen, or a mixture of nitrogen and oxygen with 5 per cent of the latter, was used, no change of gas taking place in chambers A and C, but with the gas in chambers B and D changed every 12 hours. In all cases very low rooting values were obtained, but the highest value was for the chamber through which the largest amount of oxygen passed. In spite of the total amount of oxygen inserted in chamber B, it seems probable that, due to the rusting of the iron-wire stilts, an actual deficiency of oxygen occurred in this case as well as in the other three bottles where obviously very little oxygen was available for rooting.

In every experiment referred to in Table 9 (except experiment 17) one or more chambers showed evidence of oxygen deficiency. In some cases this deficiency was brought about by an insufficient volume of air, in some cases by inadequate partial pressure of oxygen in the gas mixture, and in some cases by a temperature too high for the other conditions.

The cuttings in experiment 23, chamber A, gave the highest rooting values obtained without flowing gases. The rooting values for the cuttings in chamber B and for those in chamber C are markedly different, which may well have been due to a difference in the available oxygen supply per cutting between the chamber that contained 4,200 c. c. of air at the beginning of the experiment and the chamber that contained 2,700 c. c.

The experimental results given in Table 9 appear to support the suggestion that callusing and rooting values do not reciprocate, but rather that each process is independent of the other, being influenced by water, oxygen, and temperature. Table 9 further indicates that callusing of willow was retarded (1) when water treatment was excessive (experiment 23), (2) when oxygen supply was too great (experiments 17 and 21), and (3) when oxygen supply was too small (experiments 18, 19, 20, and 23).

No clear indications regarding the controlling external factors for lenticel-callus formation were obtained in the experiments without flowing gases. Moderate lenticel callusing occurred whether the cuttings were supplied with large or with small amounts of water or oxygen. Only in experiment 18 (where all chambers had very little oxygen) did there appear any indication that oxygen supply was a limiting factor. Here all values for this form of activity were low, and apparently the lower the oxygen supply the lower were the values obtained. Also lenticel callusing seems to have had an even lower critical limit of oxygen supply than had top or base callusing.

A preliminary experiment set up October 26, 1926, indicated, however, that even with negligible amounts of oxygen very large lenticel calluses may form in time. In this experiment the bases of the cuttings rested on damp cotton, and nitrogen was bubbled through alkaline pyrogallol solution and then through the chamber containing the cuttings. A rubber bag with nitrogen under slightly more than atmospheric pressure remained attached to the chamber, the pyrogallol bottle being between the bag and the chamber. The set-up was placed in the greenhouse, shaded from intense sunlight. After one month the cuttings showed very large lenticel calluses, but rooting was almost negligible. Neither rooting nor callusing occurred during the next 12 days. On December 8 the chamber was opened to the air, and four days later many roots were visible on each cutting, indicating that lack of oxygen in the first part of the experiment had been the chief factor to retard rooting, even though optimum conditions had been offered for lenticel-callus formation.

Few indications regarding cardinal temperatures are to be obtained from Table 9, since only a single temperature was employed in each experiment. It may be noted, however, that the highest rooting values were shown by injected cuttings kept at 29° C., while the highest values for top and base callusing are shown by cuttings kept at 32° and supplied with additional water. In general, the higher the temperature the greater the supply of oxygen necessary for optimum rooting. This seems to have been indicated only slightly for callusing at top or base, and not at all for lenticel callusing.

In an experiment carried out in April, 1926, it was observed that one lot of willow cuttings with bases in water showed the same root development as did another lot with bases embedded in a 1.25 per cent agar gel. In the light of the more recent experiments, these results suggest that either the small amount of oxygen necessary for rooting was able to diffuse fast enough through the agar, or else the necessary oxygen reached the bases of the cuttings through the freely exposed tops. *Tradescantia* cuttings treated in the same manner as the willow cuttings also rooted in agar, but with this difference—the roots formed only near the top of the gel, suggesting that more oxygen is required for its rooting than for willow.

The experiments here reported with willow cuttings without flowing gases are no more than preliminary, but the results obtained seem to strengthen some of the suggestions brought out in experiments 1 to 12; namely, (1) that for both rooting and callusing a rather definite and comparatively low supply of oxygen is necessary; (2) that the minimum oxygen supply for callusing is less than that for rooting; (3) that excess of oxygen may slightly retard rooting and markedly retard callusing; and (4) that the oxygen minimum is

greatly dependent upon the temperature. The chief difference between apple and willow as shown in these experiments seems to lie in the much higher point for the oxygen minima in the former.

SUMMARY

The experiments here reported were of a general and exploratory nature, planned to secure indications as to promising methods of procedure in both the planning and the execution of tests of rooting and callusing. Fall, winter, and spring cuttings were tested in darkness, at seven different maintained temperatures in each of 16 series with constantly flowing gas, and these series differed with respect to the oxygen and water conditions in the test chambers. Some preliminary treatments with water were tested in this way, and some experiments were tried without flowing gas. With the different maintained temperatures and with flowing gas the experiment period was 10 days, while an experiment period of 7 days was used in some of the tests without flowing gas. The plants used were apple of the Springdale variety and willow (*Salix alba* L.), both of which have preformed, dormant root primordia under and in the bark. In the Springdale apple these primordia occur in groups, forming burrknots. The apple cuttings were made from wood from 3 to 10 years old; most of the willow cuttings were made from wood 2 years old.

RESULTS WITH APPLE CUTTINGS.—For the 10-day period the temperature curve of rooting showed generally a minimum of about 8° to 14° C., a maximum of about 39°, and an optimum of about 24° to 29°; but these cardinal points of the temperature relation were apparently shifted or modified according to the oxygen and water conditions.

The maximum temperature was consistently about 39° C. in all cases where the oxygen supply was adequate. When the oxygen supply was apparently inadequate, the maximum temperature was not so high.

The optimum temperature appeared to be influenced by internal conditions related to the seasonal maturity of the shoots from which the cuttings were made. With apparently adequate oxygen and water conditions the optimum for the spring cuttings seemed to be shifted downward on the temperature scale, and it was also markedly broadened into an optimal range. The fall cuttings showed a more definite and somewhat higher optimum than did the spring cuttings, and the latter rooted equally well throughout an extensive optimal range.

The numerical values or scores representing the amount of rooting were considerably greater for the optimal temperature range in the spring than they were for the fall optima. In the later tests, with more mature shoots, at sub-optimal and supra-optimal temperatures the rooting values were greater than at optimal temperatures in the fall tests. More rooting and rooting at lower temperatures generally occurred in cuttings made and tested in March than in those made and tested in November.

The minimum temperature for rooting appeared to be decidedly lower in the spring tests than in the fall tests, suggesting that the minimum was shifted downward on the temperature scale as the shoots became more mature.

The minimum temperature for rooting did not seem to be influenced by water and oxygen conditions as these were tested.

Some evidence secured suggested that a longer experiment period would have given lower minima than those given during the 10-day period.

While the slope of the temperature curve for rooting for supra-optimal temperatures appeared to be much influenced by oxygen and water conditions, this influence was not pronounced for sub-optimal temperatures.

Neither injurious nor beneficial effects on rooting appeared from exposing the cuttings to oxygen at a pressure of about 1 atmosphere. When ordinary air was passed through the test chamber at an adequate rate, the rooting in apple cuttings was just as satisfactory as when oxygen with only slight admixture of nitrogen was used.

Rooting values were higher when the cuttings had been subjected to an initial treatment with water, but the extent of such treatment might be excessive, in which case retardation was more or less pronounced, seemingly according to the de-

gree of excess. Thus, soaking the cuttings one hour in water resulted in increased rooting, with other conditions good, but soaking them 48 hours resulted in marked retardation of rooting. On the other hand, callusing seems to have been retarded by any water treatment, this retardation being approximately proportional to the degree of water treatment.

Callusing seems to have been more active at slightly higher temperatures and with somewhat lower water and oxygen supplies than those inducing the most active production of roots.

Although the maximum temperature for callusing was about the same as that for rooting and the optimum was only slightly above that for rooting, the minimum temperature for callusing was generally from 4° to 8° above the corresponding temperature for rooting in the same experiment.

With good environmental conditions, callus formation was generally found to be more active at the base than at the top of a cutting. This suggests an internal influence of polarity on callusing, in which respect callusing seems to differ from rooting, for which no such influence was apparent.

A distinct retardation of callusing appeared when the cuttings were surrounded with oxygen at a pressure of about 1 atmosphere, but no such retardation was observed in the case of rooting.

Although both rooting and callusing were more active at the season of the year when buds are ready for development (March), this increased activity in root and callus formation was as apparent for cuttings without buds as for those with buds; thus there seemed to be no direct influence exerted by buds on either rooting or callusing.

For formation of lenticel callus some oxygen seems to have been necessary, but the amount of oxygen required for lenticel callusing was much less than for top or base callusing.

RESULTS WITH WILLOW CUTTINGS.—In general, the results with willow cuttings were similar to those with apple, with some quantitative differences apparent in the cardinal temperatures and in the oxygen and water relations. The chief points of difference shown by willow cuttings were as follows:

Although for the rooting of willow cuttings an optimum temperature of about 29° C. was indicated, similar to that shown for apple, all temperatures tested between 20° and 33° were very good for willow.

Although some seasonal variation was observed, similar to that in apple cuttings, in all cases the minimum temperature appears to have been several degrees higher for rooting of willow.

All water treatments employed seemed beneficial to willow rooting; with a greater degree of water treatment, proportionally more rooting was apparent.

The minimum oxygen supply for rooting and callusing was so low that oxygen deficiency was not evident except in some of the experiments without flowing gases.

When no water treatment was used, a large oxygen supply seemed slightly to retard rooting. There was even some suggestion that the minimum temperature might be raised by oxygen excess.

Top and base callusing seems to have been more influenced in willow than in apple by excess of oxygen.

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VITA

Charles F(letcher) Swingle was born May 13, 1899, near Manhattan, Kansas, the son of John F., and Stella (Leonhardt) Swingle. He attended the public schools in and near Manhattan, Kansas, and in Portland, Oregon. In 1920 he received the degree of Bachelor of Science in Agriculture from the Kansas State Agricultural College, having completed the general course in agriculture, with some specialization in horticulture. He was married in 1920 to Mildred C. Berry. From March 1923 until June 1927 he was a student in the Johns Hopkins University, in 1927 receiving the degree of Doctor of Philosophy, with plant physiology as his principal subject (under Professor Burton E. Livingston). The work in the first subordinate (botany) consisted largely of anatomical research under the guidance of Professor Duncan S. Johnson, represented by a paper on "Burrknot formations in relation to the vascular system of the apple stem," published in the Journal of Agricultural Research for March 15, 1927. Work for the second subordinate (genetics) consisted largely of bibliographical research done under the guidance of Professor Herbert S. Jennings, represented by a paper entitled "Graft hybrids in plants," published in the Journal of Heredity for February, 1927.

In 1918 he was engaged in commercial nursery work with citrus trees, including bud-selection, in and near Riverside, California. In 1920 and 1921 he was similarly engaged in central California, but with deciduous fruit trees, especially in budding operations. Since 1922 he has been continuously engaged in research in the field of plant propagation, in the United States Department of Agriculture.

In 1927-28 he was National Research Council fellow of the International Education Board, working at the University of Leeds (England) with Professor J. H. Priestley. The principal results of this fellowship are contained in Technical Bulletin 151 of the United States Department of Agriculture, "Vegetative propagation from the standpoint of plant anatomy." During this period he visited many commercial nurseries as well as research institutions in central Europe, attending the fifth international Genetics Congress in Berlin and the eighth international Horticultural Congress in Vienna, the latter as the American representative.

Between June and December, 1928, he was engaged in the first American botanical expedition into Madagascar, in company with Professor Henri Humbert of the University of Algiers. The chief result of this expedition was the bringing back of living material of *Euphorbia intisy* and other rubber-producing plants; a description of this trip is contained in "Across Madagascar by boat, auto, railroad, and filanzana," in the National Geographic Magazine for August, 1929.

He is the translator of Küster's Pathologische Pflanzenanatomie, which is now in process of publication in English.

